What's in a name? Using species delimitation to inform conservation practice for Chinese giant salamanders (Andrias spp.)

Article *in* Evolutionary Journal of the Linnean Society · June 2024

DOI: 10.1093/evolinnean/kzae007

9 authors, including:

Melissa Marr
University of Edinburgh
19 PUBLICATIONS **131** CITATIONS
SEE PROFILE

Kevin Hopkins
Zoological Society of London
46 PUBLICATIONS **998** CITATIONS
SEE PROFILE

Benjamin Tapley
ZSL
179 PUBLICATIONS **1,492** CITATIONS
SEE PROFILE

Amaël Borzée
Nanjing Forestry University
347 PUBLICATIONS **2,928** CITATIONS
SEE PROFILE

What's in a name? Using species delimitation to inform conservation practice for Chinese giant salamanders (*Andrias* spp.)

Melissa M. Marr[1], Kevin Hopkins[2], Benjamin Tapley[3], Amaël Borzée[4,5], Zhiqiang Liang[6], Andrew A. Cunningham[2], Fang Yan[7], Jie Wang[8] and Samuel T. Turvey[2]

[1]*Roslin Institute, University of Edinburgh, Easter Bush Campus, Midlothian, EH25 9RG, UK*

[2]*Institute of Zoology, Zoological Society of London, London NW1 4RY, UK*

[3]*Living Collections, Zoological Society of London, London NW1 4RY, UK*

[4]*Laboratory of Animal Behaviour and Conservation, College of Life Sciences, Nanjing Forestry University, Nanjing 210037, China*

[5]*Jiangsu Agricultural Biodiversity Cultivation and Utilization Research Centre, Nanjing 210014, China*

[6]*Hunan Fisheries Research Institute, Changsha 410153, China*

[7]*School of Life Sciences, Yunnan University, Kunming 650500, China*

[8]*Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China*

Corresponding author: samuel.turvey@ioz.ac.uk

Abstract. Genetically-defined biodiversity units must align with practical conservation frameworks, and most conservation is conducted at the species level. Chinese giant salamanders have traditionally been interpreted as the single widespread species *Andrias davidianus*, but molecular studies have reinterpreted this taxon as representing multiple allopatric clades, and competing taxonomic hypotheses support different numbers of candidate species. We conducted species delimitation analyses using tree-based models (General Mixed Yule Coalescent, Poisson Tree Processes) and alignment-based models (Bayesian Phylogenetics and Phylogeography) to interpret diversification across *Andrias* within a comparative systematic framework, using 30 mitogenomes representing all recognised Chinese clades. Nearly all tested models provide support for at least seven statistically-resolved Chinese species-level lineages, and most provide support for nine species. Only four species have available names. Chinese *Andrias* populations are Critically Endangered, but unnamed species cannot be incorporated into national or international conservation frameworks and risk being excluded from recovery efforts. We urge taxonomists and conservation practitioners to focus more attention on the world's largest amphibians, and non-standard taxonomic approaches may be required to name these species before they disappear.

Keywords: *Andrias davidianus*; coalescent theory; conservation; mtDNA; phylogenetic species concept; species delimitation; undescribed species; taxonomy

Introduction

Cataloguing biodiversity is crucial to inform conservation and help mitigate the global extinction crisis (Mace 2004, Costello *et al*. 2013, Thomson *et al*. 2018, O'Donnell *et al*. 2020). Although conservation policy increasingly recognizes the need to retain biodiversity across multiple dimensions (genes, populations, species, evolutionary history, ecosystems, contributions to humanity) (Díaz *et al*. 2020), the majority of practical conservation planning and management is conducted at the species level (Coates *et al*. 2018). Taxonomy therefore fundamentally underpins conservation. Without a formal description and name, a species cannot be incorporated into national or international frameworks, its extinction risk cannot be assessed, and vital conservation action often cannot take place (O'Donnell *et al*. 2020).

However, taxonomy is dynamic (Thiele *et al*. 2021): it involves both the description of biodiversity based upon available data, and decisions about how new understandings are translated into nomenclature (Zachos *et al*. 2019). Taxonomic change can arise through changes to paradigms used for defining species (Dufresnes *et al*. 2023), such as different species concepts (Freudenstein *et al*. 2017, Padial and De la Riva 2020), and through acquisition of new data, often associated with increased molecular analyses (Coates *et al*. 2018). In particular, molecular approaches have made advancements in differentiating cryptic species (multiple species previously considered conspecific due to morphological similarity) (Bickford *et al*. 2007). Changing formal taxonomic units, through either 'lumping' or 'splitting' of pre-existing species categories, has implications for conservation (May 1990, Gutiérrez and Helgen 2013, Garnett and Christidis 2017), especially if practitioners fail to translate evidence for genetically-defined units into species categories that can be incorporated into real-world planning and legislation (Coates *et al*. 2018). There thus exists a serious risk of misalignment between scientific hypotheses of threatened biodiversity, and formal biodiversity frameworks used for making decisions and allocating resources in conservation (Ely *et al*. 2017, Garnett and Christidis 2017, Thomson *et al*. 2018, O'Donnell *et al*. 2020).

Misalignments between taxonomic hypotheses and conservation can be addressed using statistically rigorous species delimitation models, which can improve the consistency, objectivity and stability of defining species boundaries across related groups using genetic datasets. These methods are most powerful when applied to multi-locus data (Fujita *et al*. 2012, Carstens *et al*. 2013). However, when target species are rare or represented only by poorly-preserved archival specimens or degraded DNA, available data are often limited to single mitochondrial DNA (mtDNA) loci or whole mitochondrial genomes (mitogenomes) (Wandeler *et al*. 2007, Barnes and Turner 2015). Several methods are available that perform well with mitogenome data, including coalescent theory and Poisson Tree Processes (PTP) models. Coalescent theory is a widely-used statistical framework for modelling the stochastic process of how lineages merge over time given demographic parameters (Kingman 2000, Fujita *et al*. 2012), and is incorporated into methods such as the General Mixed Yule Coalescent model (GMYC; Pons *et al*. 2006, Fontaneto *et al*. 2007, Fujisawa and Barraclough 2013) and Bayesian Phylogenetics and Phylogeography (BPP; https://github.com/bpp/bpp). PTP models estimate transitions in branch lengths below and above the species level, modelling speciation and coalescence events as Poisson processes along branches (Zhang *et al*. 2013).

Giant salamanders (Cryptobranchidae: *Andrias*), the world's largest amphibians, have traditionally been interpreted as comprising two extant species, the Chinese giant salamander *Andrias davidianus* (Blanchard, 1871) and Japanese giant salamander *A. japonicus* (Temminck, 1836). All giant salamanders are threatened: *A. japonicus* is assessed as Vulnerable on the IUCN Red List, and *A. davidianus* has been assessed as Critically Endangered since 2004 (IUCN SSC Amphibian Specialist Group 2023a) and in Chinese national legislation since 1989 (Zhao 1998, Wang *et al*. 2004). Chinese populations have declined severely across their historical range, largely due to emergence of a massive-scale commercial farming industry to supply the domestic luxury food market, which has driven illegal harvesting of wild individuals to supply breeding stock (Cunningham *et al*. 2016, Turvey *et al*. 2018, Tapley *et al*. 2021, Turvey *et al*. 2021). A multi-year survey of 97 sites with suitable predicted giant salamander habitat and/or historical records, conducted across 16 Chinese provinces in 2013-2016, only detected salamanders at four sites, and all of these animals might have originated from nearby farms rather than representing wild individuals (Turvey *et al*. 2018). Remnant wild populations have subsequently been located at a few sites (Wang *et al*. 2017, Liang *et al*. 2019, Chai *et al*. 2022).

Molecular studies conducted over the past two decades using different methods (isozymes, mtDNA genes, microsatellites, single nucleotide polymorphisms (SNPs)) have consistently concluded that giant salamanders across central, eastern and southern China represent genetically distinct local populations (Murphy *et al*. 2000, Tao *et al*. 2005, Wang *et al*. 2017). Large-scale genetic analysis of wild-caught and farm-bred individuals using mtDNA loci and SNPs identified between five and seven distinct clusters (referred to as mtDNA haplotype clades A-E and U1-U2); these clades could not be consistently associated with specific landscapes, as recent human movement of individuals for farming had obscured historical patterns of biogeography (Yan *et al*. 2018). The same seven clades were recovered by Liang *et al*. (2019), using more extensive mtDNA data (including 20 mitogenomes) sampled from reportedly wild-caught adults and larvae, although with a slightly different pattern of phylogenetic relationships (clades referred to as A-G; see Table 1 for correspondence between two sets of clade names). Associated locality data demonstrated that clades exhibited largely non-overlapping allopatric distributions across different watersheds and montane ecoregions, although with some spatial mismatches probably associated with inclusion of translocated individuals within the study.

Whereas both Yan *et al*. (2018) and Liang *et al*. (2019) inferred a deep divergence between all clades (late Miocene to middle Pliocene, 11.1-3.7 Mya), their taxonomic status was left unresolved: they were referred to as 'species lineages' (Yan *et al*. 2018) or intraspecific *A. davidianus* populations (Liang *et al*. 2019), and remained undescribed and unnamed. Clade B (Yan *et al.*)/clade G (Liang *et al.*) corresponds to *A. davidianus* sensu stricto, based upon holotype collection locality (Liu 1950, Dai *et al*. 2009, Turvey *et al*. 2019). Analysis of partial or near-complete mitogenomes from historical samples by Turvey *et al*. (2019) was able to associate a museum specimen described in the early twentieth century as a distinct species, *Andrias sligoi* (Boulenger, 1924), with clade D (Yan *et al.*)/clade B (Liang *et al.*), and assigned this species name to the clade. This species was recently assessed as Critically Endangered (IUCN SSC Amphibian Specialist Group 2023b). More recently, a wild population of clade U2 (Yan *et al.*)/clade D (Liang *et al.*) has been described as the new species *A. jiangxiensis* Lu, Wang, Chai, Yi, Peng, Murphy, Zhang and Che in Chai *et al*. (2022). A further species, *A. cheni* Xu, Gong, Li, Jiang, Huang and Huang in

Gong *et al*. (2023), has been described from Huangshan, the source of samples assigned to clade E (Yan *et al*. / Liang *et al*.). Other clades remain undescribed, and the taxonomic status of the Chinese *Andrias* radiation is unclear in light of the competing hypotheses proposed by Yan *et al*. (2018) and Liang *et al*. (2019) and a lack of wider comparative assessment of phylogenetic patterns and evolutionary dynamics across related taxa.

Phylogeographic structuring of *Andrias* matrilines across China indicates that conservation management frameworks need to recognise local genetic differentiation of giant salamander populations. Mixing of animals in farms, and well-intentioned releases of farm-sourced animals translocated from different regions of China, is understood to pose a severe threat through hybridisation and ecological replacement of remnant populations of local clades, in addition to risk of disease transfer (Yan *et al.* 2018, Turvey *et al*. 2018, Turvey *et al*. 2019, Shu *et al*. 2021). China's Aquatic Wildlife Conservation Association has recently acknowledged the existence of at least five Chinese giant salamander species, and recommends that releases without genetic screening should be prohibited (CAWCA 2023). However, China's national List of Wildlife under Special State Protection, which was substantially revised in 2021 for the first time since 1988, still only includes one named Chinese giant salamander species, *A. davidianus*. Exclusion from this state protected species list is known to impede effective conservation (Ping and Zeng 2020). The IUCN Red List also currently provides extinction risk assessments for only *A. davidianus* and *A. sligoi* (IUCN 2024).

A key step to guide conservation policy for Chinese giant salamanders is to critically evaluate whether genetic differentiation across Chinese *Andrias* populations should be interpreted as multiple distinct species or intraspecific diversification. If allopatric clades are demonstrated to represent species-level differentiation, it is then necessary to determine what options are available to formally name the undescribed species and integrate them within conservation planning frameworks, and to determine which options are most feasible and appropriate in light of the perilously rare and threatened status of all wild populations. We address these urgent questions by conducting species delimitation analyses of available genetic data for *Andrias* clades representing discrete candidate species. Our findings provide a new baseline for interpreting, defining and conserving species diversity in Chinese giant salamanders, and our study has wider relevance for incorporating cryptic species as conservation units into real-world policy and management.

Materials and Methods

Data and phylogenetic reconstruction

To test whether geographically-structured matrilineal clades observed within Chinese *Andrias* should be interpreted as distinct species, species delimitation analyses were conducted using 20 mitogenomes from recent field-collected samples reported by Liang *et al*. (2019), combined with nine mitogenomes from historical specimens reported by Turvey *et al*. (2019) and one mitogenome from Xu *et al*. (2016). These samples cover all seven previously-identified Chinese clades (samples per clade: 1-10; Table 1, Table S1).

Partial and full-length mitogenomes were obtained from GenBank for the amphibian suborder Cryptobranchoidea (comprising the families Cryptobranchidae and Hynobiidae). Taxonomic conventions and placements follow the Amphibian Species of the World list (https://amphibiansoftheworld.amnh.org) and the IUCN Red List (2024). Three alignments were created to reconstruct mitogenome phylogenies: (1) 30 Chinese *Andrias* sequences only; (2) Chinese *Andrias* sequences and one *A. japonicus* sequence as outgroup ('all-*Andrias*'); (1) 140 available sequences for the Cryptobranchoidea, comprising all *Andrias* sequences and 39 species of Hynobiidae (pruned to remove excessive duplicates; Table S1).

For each separate dataset, an alignment was performed using Muscle v.3.18 (Edgar 2004) in the Geneious Pro v.8 platform (Kearse *et al*. 2012). Sequence annotations, determined using MITOS v.2.1.8 (Donath *et al*. 2019) and GenBank information, were used to extract and concatenate the two rRNAs, 22 tRNAs and 13 protein-coding genes per alignment. The Chinese *Andrias* alignment did not require a substitution model as it was used for network-building on uncorrected p-distances. The other alignments were submitted separately to PartitionFinder2 (Lanfear *et al*. 2017), which was used to establish optimal partitioning strategies and best-fit nucleotide substitution models. This was run using models available in MrBayes v.3.2.7 (Ronquist *et al*. 2012) and applying AICc model selection criteria and the 'rcluster' search parameter (Lanfear *et al.* 2014). This approach identified the GTR+G substitution model as optimal for the all-*Andrias* alignment, and the GTR+G+I substitution model for the Cryptobranchoidea alignment (likely due to added complexity resulting from inclusion of diverse hynobiid data).

Different tree-building approaches were implemented for the all-*Andrias* and Cryptobranchoidea alignments. For *Andrias*, a Bayesian phylogeny was generated in MrBayes v.3.2.7 (Ronquist *et al*. 2012) with *A. japonicus* as outgroup. Four chains were run for 2×10^6 generations, sampling every 100 iterations, with run performance and chain convergence assessed using summarised run statistics; a 50% consensus tree was generated after 25% of trees were discarded as burn-in. The same alignment was used to create an uncalibrated maximum likelihood (ML) phylogeny for *Andrias* in RAxML v.8 (Stamatakis 2014) with a majority-rule tree as the output phylogeny and with 1000 bootstrap replicates. A neighbour-net phylogenetic network was also generated for Chinese *Andrias* using SPLITSTREE v.5.3.0 (Huson and Bryant 2006), using least-squares variance, uncorrected p-distances, heterozygous ambiguities averaged and normalized, and the equal angle algorithm, with branch support estimated using 1000 bootstrap replicates.

A phylogeny for the Cryptobranchoidea was reconstructed using BEAST v.2.6.4 (Bouckaert *et al*. 2019) for use with a GMYC model. This approach performs optimally on a complete phylogeny and requires a time-calibrated ultrametric tree (Fujisawa and Barraclough 2013), so calibration points were derived from molecular, fossil and geological data (Table S2; see Turvey *et al*. 2019). The oldest putative crown-group cryptobranchid, the Middle-Upper Jurassic (Bathonian-Oxfordian) *Chunerpeton tianyiense* (Gao and Shubin 2003), is often used to constrain the origin of Cryptobranchoidea (e.g. Zhang *et al*. 2006, Zhang *et al*. 2008). However, *Chunerpeton* has recently been referred to the stem-Caudata and thus falls outside the Cryptobranchoidea (Rong *et al.* 2021). Turvey *et al*. (2019) used *Chunerpeton* to constrain the origin of Cryptobranchoidea as the mean of the time range between start-Bathonian and end-Oxfordian, with soft bounds including all of this range (162.8±5.5 Mya). In the absence of an alternative fossil calibration point, we retain a Middle Jurassic mean origin, but with generous soft bounds that extend through the Early to Late Jurassic (168±20 Mya). The Cenozoic cryptobranchid *Aviturus exsecratus* (Gubin 1991, Vasilyan and Böhme 2012) was used to constrain minimum age of the Cryptobranchidae at 56 Mya. Molecular estimates of divergence times derived from nuclear genes were used to constrain the origin of crown-group Hynobiidae (mean age: 135.1 Mya, soft bounds: 120-

151 Mya), and the timing of major diversification within the family (i.e. excluding *Onychodactylus* spp.; mean age: 40.2 Mya, upper and lower bounds: 34.5-46.2 Mya; Chen *et al*. 2015). The timing of geological isolation of Japan from mainland Asia (Isozaki *et al*. 2010) was used to constrain a mean origin for *Andrias* of 16 Mya (upper and lower bounds: 14.5-17.6 Mya). Priors and calibrations for the estimated most recent common ancestor of the Chinese *Andrias* radiation were obtained from Liang *et al*. (2019). The dataset used an uncorrelated log-normal clock, a Yule tree prior using a random starting tree, and the model of nucleotide substitution suggested by PartitionFinder2 (GTR+I+G). More complex analyses using partitions and birth-death priors were explored but tended to result in insufficient sampling and convergence. The analysis was run for 3.5×10^{8} generations, sampling from the prior every 3500 generations. TRACER v.1.7.2 (Rambaut *et al*. 2014) was used to assess chain convergence and sufficient sampling in the .log file before trees were resampled in LogCombiner, to provide a final set of 10,000 trees. A Maximum Clade Credibility (MCC) tree was then created in TreeAnnotator v.1.10 (Bouckaert *et al*. 2019) after removing 25% of trees as burn-in.

Tree-based species delimitation

GMYC and PTP approaches were used to delimit operational taxonomic units in *Andrias* based on the phylogenetic species concept (PSC) and using a gene tree as input.

GMYC is a tree-based likelihood method for species delimitation, which models switches between speciation events and intraspecific coalescence events in the branching patterns of a time-calibrated ultrametric tree by optimizing a ML solution (Pons *et al*. 2006, Fontaneto *et al*. 2007, Fujisawa and Barraclough 2013). It uses a Yule model for interspecific branching events that assumes no extinction and a constant speciation rate (Nee *et al*. 1994), and a neutral coalescent process to model intraspecific branching events (Hudson 1990). We used both the single-threshold model (sGMYC), which assumes a single threshold time-shift between species diversification and intraspecific coalescences (Pons *et al*. 2006, Tomochika and Barraclough 2013), and the Bayesian model (bGMYC), which samples from a posterior distribution of ultrametric trees and pools Markov-Chain Monte-Carlo (MCMC) runs per tree to generate posterior probabilities for sampled species (Reid and Carstens 2012). The multiple-threshold GMYC model was not used as it can overestimate species numbers and performs poorly in comparison to sGMYC (Fujisawa and Barraclough 2013). For sGMYC analysis, the R package 'splits' v.1.0 (Ezard *et al*. 2009) in R v.4.1.1 (R Core Team 2021) was used to perform species delimitation using the BEAST MCC tree as the input phylogeny. For bGMYC analysis, LogCombiner was used to resample the 10,000 BEAST trees to extract 100 trees from the posterior distribution after 25% burn-in. MCMC was run for 50,000 generations, with a burn-in of 40,000 and thinning of 100. Parameters t1 and t2 were set to t1=2 and t2=140. The fit between data and models was assessed using P2C2M.GMYC with ML parametric bootstrapping (Fonseca *et al*. 2020).

PTP looks for significant differences in substitution rates among and within species rather than identifying transitions in branching rates, so does not require a time-calibrated ultrametric phylogeny. Both ML and its Bayesian implementations (bPTP; Stamatakis *et al*. 2013) were run for PTP analysis. To examine the effect of phylogeny on species delimitation performance of PTP, the all-*Andrias* Bayesian tree and the Cryptobranchoidea BEAST MCC tree were both used as input phylogenies for PTP and bPTP. Analyses were conducted using the bPTP server (https://species.h-its.org; Zhang *et al*. 2013) with

250,000 generations, thinning set to 250, and burn-in of 25%. Model convergence was determined by visual inspection of MCMC iterations against log likelihoods.

Sequence alignment-based species delimitation

Joint species delimitation and species-tree estimation (analysis A11) was performed in BPP v.4.4.1 (https://github.com/bpp/bpp). In contrast to tree-based methods, BPP performs its analyses on sequence alignments within a Bayesian framework using a reversible-jump Markov Chain Monte Carlo search to test between species delimitation models (Yang 2002, Rannula and Yang 2003, Flouri *et al*. 2018). The multispecies coalescent model (Xu and Yang 2006) infers boundaries of closely related species (<10% divergence) by comparing delimitation models and estimating the sum of the posterior probability of species trees, and is often applied to mitogenomes. As limited information is available to inform priors set for extant and ancestral population size (θ) and species divergence times (τ), a diffuse prior (α=3) was set for population size parameters and root divergence time, following Flouri *et al*. (2020). The shape parameter for the gamma distribution of population size (β) was adjusted to give a range of means representing high, mid-range and low values to explore the effects of priors on species delimitation, with seven prior combinations used, and each run repeated twice on a different starting seed to ensure convergence on the same posterior (Table 2). The MCMC chain was run for 250,000 steps, with a sampling frequency of 5, following burn-in of 50,000. Samples were assigned to putative species based on which mtDNA clades they were assigned to in phylogenetic reconstructions. As BPP does not split species, the maximum number of putative species suggested by other delimitation methods and phylogenetic analyses (n=9) was used to define maximum possible species number.

Congruence between mitogenome and gene trees

To explore whether useful genetic markers could be identified for rapid taxonomic assignment of *Andrias* individuals, further investigation was conducted to assess whether the clades reconstructed in the whole-mitogenome phylogeny could be recovered using shorter mtDNA sequences. Gene regions previously used to investigate Chinese *Andrias* phylogenetics were trimmed from the BEAST input mitogenome alignment. Three single-gene regions were investigated: cytochrome oxidase 1 (Yan *et al*. 2018), D-loop (Tao *et al*. 2005), and cytochrome *b* (Tao *et al*. 2005). Two combined multi-gene samples were also investigated: a combined three-gene consensus tree (comprising all three single-gene regions), and the partial cytochrome *b* to control region fragment investigated by Liang *et al*. (2019). Trimmed alignments were used as inputs for simple neighbour-joining gene trees built using the PAUP plugin for Geneious v.7.1.9 (https://www.geneious.com). The cytochrome *b* gene was also used as input for a comparative Bayesian gene tree built using the MrBayes 3.2 plugin in Geneious (parameters used: substitution model HKY85; chain length 1,100,000; subsampling frequency 200, with burn-in of 100,000).

Results

Sequence alignments and phylogenetic reconstruction

Final sequence lengths after alignment and trimming were 15,437 base pairs (bp) for Chinese *Andrias* sequences (n=30), 15,443 bp for all-*Andrias* sequences (n=31), and 15,818 bp for Cryptobranchoidea sequences (n=140). Within the Chinese *Andrias* alignment, there were 7152 sites with gaps or missing data, 7914 monomorphic sites, and 376 polymorphic sites. The number of haplotypes were 25, with haplotype diversity (Hd) = 0.98 and nucleotide diversity (π) = 0.008. The network (Fig. 1) and the ML and Bayesian trees (Fig. 2) all yielded well-supported phylogenies with identical topologies, suggesting a minimum of seven monophyletic groupings matching the seven clades reported by Yan *et al*. (2018) and Liang *et al*. (2019); these clades are referred to as A-G (cf. Liang *et al*. 2019). Network bootstrap support for these divisions was 100 for all groups, and branch support in trees was high: ML bootstraps were >98, and Bayesian posterior probabilities were 1 (Fig. 2).

The time-calibrated BEAST tree recovered a highly-supported phylogeny for Cryptobranchoidea (Fig. S1). Chains showed good convergence and all ESS values were >200 (Table S3). The most recent common ancestor dates for major nodes were: (a) tree root = 155.0 Mya (95% HPD: 135.7-173.8 Mya); (b) Hynobiidae = 125.0 Mya (95% HPD: 109.6-140.6 Mya); (c) Hynobiidae excluding *Onychodactylus* = 58.6 Mya (95% HPD: 50.3-67.1 Mya); (d) Cryptobranchidae = 61.3 Mya (95% HPD: 56.0-70.8 Mya). Divergence between Chinese and Japanese *Andrias* species was estimated at 15.8 Mya (95% HPD: 14.0-17.5 Mya). Node date estimates for Chinese *Andrias* clades are shown in Fig. 3. The tree was heavily calibrated to provide a phylogeny reflective of known divergence dates, and thus generate branching patterns suitable for GMYC analysis; node ages may therefore be influenced more by calibration designations than sequence data.

Tree-based species delimitation

Across the Cryptobranchoidea, sGMYC results rejected the null model that all sequences belong to one species (likelihood value of null model = 164.64, likelihood of sGMYC model = 204.51, likelihood ratio = 79.14, p<0.01), and delimited 56 species (maximum likelihood entities) comprising 18 clusters (95% CI: 17-19) (Table S4). Within the Chinese *Andrias* samples, sGMYC delimited seven highly-supported species corresponding to clades A-G (Fig. 3), with branch support of 1 for all clades with >1 sequence (Fig. S2; node support for single sequences cannot be calculated).

P2C2M.GMYC and bGMYC produced conflicting results when assessing suitability of GMYC to the *Andrias* dataset. P2C2M.GMYC suggested the data did not violate GMYC assumptions (p=0.8), whereas bGMYC indicated uncertainty in the species boundaries suggested by sGMYC and gave a conservative estimate of species richness: Clade A was delimited with 0.99 posterior probability, clade B+C with 0.85 posterior probability, and Clade G with 0.78 posterior probability, whereas other clades were delimited with 0.1-0.6 posterior probability (Figs 3-4).

PTP and bPTP analyses were identical for both the all-*Andrias* and Cryptobranchoidea trees, so only the bPTP results are reported. MCMC iterations and log-likelihood scores showed good convergence for both trees (Fig. S3), and PTP and bPTP produced identical species groupings and support values (Fig. 5, Table 3). The estimated number of species in the all-*Andrias* tree was 7-15 with a mean of 10.15 (acceptance rate: 0.182). The bestsupported species delimitation was for nine Chinese *Andrias* species: clades A-G were recovered, with further subdivision of clades D and G into two further species pairs, although support for subdivision of clade G was low (clade support: G1=0.68, G2=0.37; Fig. 5, Tables 1, 3). The estimated number of Chinese *Andrias* species in the Cryptobranchoidea BEAST phylogeny was seven (species range for entire phylogeny: 55-63; mean: 58.49; acceptance rate: 0.066). This analysis did not detect further subdivision of clades D and G into additional species (Fig. 5, Table 3).

Sequence alignment-based species delimitation

All pairs of prior combinations run on Chinese *Andrias* sequences showed identical results. Runs 1-5 supported subdivision into nine species, corresponding to clades A-G and subdivision of clades D and G into additional species pairs as in PTP analyses. All posterior probabilities on these nine species were >0.9, with posterior probabilities >0.99 in runs 1 and 2 (Table 4). In runs 6 and 7, which had a θ of IG(3,0.02), species delimitation ranged between seven and nine species: the seven-species scenario replicated clades A-G, the eight-species scenario subdivided Clade G, and the nine-species scenario subdivided clades D and G (Table 4). In these runs, the eight-species scenario had highest posterior support in each run, followed by the nine-species scenario and seven-species scenario; however, posterior probabilities for these delimitations were low (0.2-0.5).

Congruence between mitogenome and gene trees

Of the single-gene regions investigated for rapid taxonomic assignment of Chinese *Andrias* individuals, the cytochrome *b* neighbour-joining tree recovered all nine distinct clades in the mitogenome phylogeny, although tree topology was not identical, and clade support was variable (54-100). Trees based upon the two combined multi-gene samples (three-gene consensus tree, cytochrome *b* to control region fragment) also recovered all nine clades. The cytochrome oxidase 1 and D-loop single-gene neighbour-joining trees and the Bayesian cytochrome *b* tree contained polytomies and did not recover all clades (Figs S4. S5).

Discussion

Validity of species delimitation analyses

By conducting tree-based and alignment-based species delimitation analyses on mitogenome data across the Cryptobranchoidea, our study provides a new baseline for interpreting diversification observed across the Chinese *Andrias* radiation within a comparative systematic framework. Our dataset is representative of those typically available for threatened species, where sample sizes can be low due to rarity and difficulties in obtaining samples.

Although we analysed mitogenomes with sequence lengths of >15,000 bp, mitogenomes represent a single locus in these analyses, and our study was restricted to a relatively small number of sequences available per putative *Andrias* species. Single-locus data can risk obscuring species-coalescence boundaries through gene tree-species tree discordance (incomplete lineage-sorting; Maddison and Knowles 2006, Mallo and Posada 2016). Importantly, mitochondrial-only data may not reflect variation shown by nuclear DNA, for instance in situations with mtDNA replacement (Dufresnes *et al*. 2019, Tamashiro *et al*. 2019), and mito-nuclear discordance is documented in other amphibians (Denton *et al*. 2014, Firneno Jr *et al*. 2020, Shu *et al*. 2022). We were restricted to mitochondrial-only analyses as we needed to incorporate data for historical museum specimens, including the *A. sligoi* holotype, for which nuclear data are unavailable due to poor archival sample quality (Turvey *et al*. 2019). We recognise that taxonomic conclusions based solely upon mitogenomic data may not necessarily be robust (Leaché and McGuire 2006, Marshall *et al*. 2021), and it is crucial to test our phylogenetic hypotheses using nuclear gene and SNP data when such comparative datasets become available for samples of taxonomic and biogeographic importance.

Despite these important constraints, we were able to recover a highly-supported tree that matched the topology of previously recovered mitochondrial and nuclear gene trees for the Cryptobranchoidea (Zhang *et al*. 2006, Zheng *et al*. 2011, Chen *et al*. 2015, Pan *et al*. 2019), and was congruent with previous dating of key nodes across this clade based upon mitochondrial and nuclear data (Zhang *et al*. 2006, Li *et al*. 2011, Chen *et al*. 2015, Shen *et al*. 2016). Our results also match the topology of previous mitogenome trees for Chinese *Andrias* (Liang *et al*. 2019). Importantly, although analysis of 772 bp RAG2 nuclear sequence data by Liang et al. (2019) did not show geographic structure across allopatric Chinese *Andrias* samples, analysis of 23,159 SNPs by Yan et al. (2018) identified the same geographically distinct clusters of wild-caught individuals that were recovered in their mitochondrial analyses. These independent lines of evidence therefore provide support for our recognition of multiple Chinese *Andrias* species-level clades, and demonstrate that restricted sample sizes available for many threatened taxa can still generate important insights about patterns of diversity, provided that a comprehensive comparative assessment is conducted across different species delimitation methods and with the strengths and weaknesses of each approach considered in their interpretation.

Taxon sampling within the Cryptobranchoidea is uneven; fewer than 50% of described hynobiid species have available mitogenomes, and considerable cryptic species diversity probably remains undescribed (Pan *et al*. 2019). Future genetic sampling of additional hynobiid taxa might therefore influence species delimitation across the group. However, recognition of additional cryptic hynobiid species would probably reduce the comparative threshold for delimiting species more widely across the Cryptobranchoidea, so would not conflict with our recovery of at least seven Chinese *Andrias* species. We also recognise the effect that the changing interpretation of whether *Chunerpeton tianyiense* represents a crown-group cryptobranchoid can have on divergence depth estimation (Rong *et al*. 2021), although we accommodate uncertainty around phylogenetic placement of this taxon by using broad soft bounds within our ultrametric tree that span much of the Jurassic. Indeed, stem-group hynobiids have recently been identified from the Middle and Late Jurassic (Jia and Gao 2019, Jia *et al*. 2021). Furthermore, variation in calibration points for the tree root would affect divergence estimates across all taxa within the tree, rather than affecting comparative divergences and species delimitation estimates specifically within *Andrias*.

Our methods are underpinned by the PSC. This species concept emphasises diagnosable evolutionary independence as the primary criterion for species delimitation, and defines a species as a reciprocally monophyletic group of populations that share derived characteristics and have a common evolutionary history that distinguishes them as a discrete entity (Nixon and Wheeler 1990, Davis and Nixon 1992, de Queiroz 2007). Morphologically cryptic species are common in amphibians (Wells 2007), and the PSC is a powerful framework for elucidating hidden species diversity from genetic data when appropriate thresholds are set. Although Chinese *Andrias* clades are able to hybridise, they also hybridise with Japanese giant salamanders, which are uncontroversially recognised as a distinct species (Fukumoto *et al*. 2015, Hara *et al*. 2023). Indeed, many well-defined divergent amphibian species are incompletely reproductively isolated and can hybridise following human-caused change to their distribution or ecology (Nadachowska 2010, Canastrelli *et al*. 2017, Borzée *et al*. 2020). We recognise that the emphasis of the PSC on phylogenetic distinctiveness fails to accommodate the additional importance of localised adaptive variation and reproductive isolation associated with Evolutionarily Significant Units below the species level (ESUs; Moritz *et al*. 1994), and we recommend further exploration of Chinese *Andrias* using genome-wide SNP datasets to better understand the potential existence of ESUs that may also warrant conservation recognition.

Only one complete mitogenome sequence was available for clades A, C and F, which could have contributed to these clades appearing distinct in our analyses. We recognise the importance of including multiple samples, ideally from different intraspecific populations, when conducting mtDNA-based taxonomy (Tamashiro *et al*. 2019). The PSC is sensitive to gene tree discordance and incomplete lineage sorting, which can be further confounded by limited taxon sampling or sequence availability (Lim *et al*. 2012). However, Yan *et al*. (2018) and Liang *et al*. (2019) both included multiple sequences for these clades, and recovered comparable phylogenetic relationships and divergence times.

Species diversity and evolution of Chinese Andrias

Nearly all species delimitation methods defined a minimum of seven Chinese *Andrias* species using mitogenomic data, with high node support and statistical significance based upon *p*-values (Figures 2, 3, 5, Tables 3, 5), albeit with discordance within and between analyses regarding the maximum number of species. The most conservative estimates were produced by sGMYC, which supported this seven-species scenario, and bGMYC, which indicated uncertainty around many species boundaries and delimited few species with high support. The poor performance of the dataset in bGMYC is probably due to topological differences between trees sampled from the posterior distribution of the BEAST2 output; conversely, sGMYC and P2C2M.GMYC use a single input tree and are not affected by such differences (Reid and Carstens 2012, Tomochika and Barraclough 2013). Conversely, most analyses (PTP, bPTP, and most BPP runs) instead provided strong support for a nine-species scenario, with clades D and G each subdivided into further species pairs (Table 1).

Phylogenetic reconstruction using mitochondrial data alone can be insufficient to delimit species accurately due to the potential for mito-nuclear discordance, and our results will require future testing with suitable nuclear data to ensure they still hold. However, our wider cryptobranchoid tree is congruent with tree topology and dating previously recovered in nuclear studies; available nuclear evidence also indicates the existence of multiple Chinese *Andrias* species; and four of the Chinese *Andrias* clades we recognise are already named as distinct species (*A. cheni*, *A. davidianus*, *A. jiangxiensis*, *A. sligoi*). This leaves the question of how many additional species-level clades should also be recognised within this radiation.

We therefore recognise at least seven Chinese *Andrias* species, and probably nine species. Mean divergence-date estimates across these nine putative species range between 9.8 and 1.0 Mya. It is estimated that half of extant amphibian species diverged from their closest extant relatives within the past 7.43 million years, and 578 species (95% credible interval: 508-628) diverged within 1 Mya (Jetz and Pyron 2018), providing further comparative support for recognising even the candidate species-pairs within clades D and G as potential species. Similar relatively recent evolutionary divergence is also seen across other eastern Asian cryptobranchoid species (Yang *et al*. 1997, Lu *et al*. 2012, Suk *et al*. 2019).

Chinese amphibian diversity hotspots are primarily in montane ecoregions (Chen and Bi 2007, Hu *et al*. 2012, Li *et al*. 2012, Li *et al*. 2018). Speciation within Chinese *Andrias* was probably driven by vicariance associated with regional Neogene orogenic activity (Turvey *et al*. 2019), with estimated diversification following the most intense period of uplift of the Tibetan Plateau (Zheng *et al*. 2000, An *et al*. 2001), and with *Andrias* clades associated with different mountain systems that formed within the past few million years (Li *et al*. 1991, Huang *et al*. 2002). Speciation in several other high-elevation cryptobranchoid and salamandroid genera across the same region is also most consistent with a series of dispersals across different mountain systems followed by isolation and divergence during the past few million years, driven by orogenesis of the Tibetan Plateau and climatic and ecological shifts associated with Pleistocene glacial cycling (Lu *et al*. 2012, Yuan *et al*. 2022).

Clades D and G display the broadest native distributions of sampled *Andrias* individuals across China (Fig. 6), and their newly-recognised species pairs are locally restricted to discrete montane ecoregions across these wide geographic areas: the eastern and western Nanling Mountains in central Jiangxi (clade D1) versus northern Guangdong (D2), and the uplifts surrounding the southwestern Sichuan Basin in the upper Yangtze watershed (clade G1) versus the Qinling orogenic belt in southern Shaanxi, southern Shanxi and northern Henan in the Yellow River watershed (clade G2). These regions all constitute centres of diversification and Quaternary habitat refugia for Chinese biota, and are recognised as local hotspots of endemism in many groups (López-Pujol *et al*. 2011a, López-Pujol *et al*. 2011b, Tian *et al*. 2018). Other locally endemic caudatan and anuran species also occur in each region (Wu *et al*. 2012, Wang *et al*. 2013, Li *et al*. 2014, Wang *et al*. 2014, Yuan *et al*. 2014, Fei and Ye 2017, Fu *et al*. 2022). Recognition of the D1-D2 and G1-G2 *Andrias* species pairs as valid species is thus supported by phylogeographic patterns of local allopatry in other regionally-occurring amphibians.

Clades D and G both have formal species names: *A. jiangxiensis* (clade D) and *A. davidianus* (clade G) (Turvey *et al*. 2019, Chai *et al*. 2022). The *A. jiangxiensis* holotype is from Ji'an, Jiangxi (Chai *et al*. 2022), the same locality as a mitogenome in our analysis referred to clade D1. Clade D2 therefore remains unnamed. The *A. davidianus* holotype was collected in Zhongba, Jiangyou, Sichuan (Liu 1950, Dai *et al*. 2009). Attempts to extract ancient DNA from the >100-year old holotype have been unsuccessful (Turvey *et al*. 2019), so this specimen cannot be assigned to any Chinese *Andrias* clade using genetic data. Wild giant salamanders are probably now extirpated from Zhongba (Dai *et al*. 2009), meaning that no further individuals can be collected to allow screening of modern samples. However, Zhongba is situated at the western edge of the Sichuan Basin, only ~200 km from the collection localities of *Andrias* samples within the same contiguous ecoregion that are assigned to clade G1 (Fig. 6), whereas clade G2 is distributed >400 km away in the eastern Qinling Mountains, a separate montane system. The only available wild-caught individual of clade F, the other clade reported from the northern boundary of central China's subtropical zone, was also collected in the Qinling Mountains, at the western end of the orogenic system. We therefore propose that the species name *Andrias davidianus* (Blanchard, 1871) should be associated with clade G1, meaning that clade G2 is currently unnamed.

How can we establish names for Chinese giant salamander species?

We recommend that all nine Chinese *Andrias* clades recognised in our study should receive formal taxonomic descriptions and names, so they can be incorporated into relevant prioritisation and management frameworks, to enable conservation planning to be applied separately for each species. Three definite Chinese *Andrias* species and two probable further species remain unnamed. These taxa include clade G2, which was previously referred to *A. davidianus*, and represents the population present in southern Shaanxi, where the giant salamander farming industry developed in the 2000s (Cunningham *et al*. 2016). This unnamed species has been moved extensively around China to stock farms (Fig. 6), and has been released or escaped into the wild across the native ranges of many other *Andrias* species, including *A. davidianus* sensu stricto (Turvey *et al*. 2018, Yan *et al*. 2018, Turvey *et al*. 2019). We therefore recommend that individuals referred to clade G1 must be managed separately from translocated farm stock.

Giant salamanders are one of several threatened large charismatic vertebrate taxa now known to comprise multiple species (Hekkala *et al*. 2011, Stewart 2013, Murray *et al*. 2019, Coimbra *et al*. 2021). Molecular methods play an increasing role in delimiting such species and understanding evolutionary relationships (Vogler and Monaghan 2007), and have recently been used to reveal unexpected diversity in other highly threatened groups (e.g. Gu *et al*. 2023). However, molecular data are typically used only to inform taxonomic conclusions (Ellepola *et al*. 2021), and new species of amphibians and other taxa are not routinely described using molecular data alone (Streicher *et al.* 2020, Dubois *et al*. 2021). Instead, taxonomists typically use an integrative approach when describing species; this is particularly important when delimiting cryptic species within morphologically conservative species complexes, which can be fraught with difficulty (Köhler *et al*. 2005, Vieites *et al*. 2009, Catenazzi 2015).

However, in situations where molecular evidence supports species delimitation, it can be extremely challenging to gather additional lines of evidence needed to describe these species appropriately. With *Andrias*, we currently have minimal understanding of morphological variation within and across Chinese populations. Previous large-scale genetic studies were mainly based on genetic swabs obtained from animals on salamander farms or from wild-caught animals that were released after sampling, with no accompanying morphological information recorded and individuals typically unable to be relocated for further investigation (Yan *et al*. 2018, Liang *et al*. 2019). Physical adult specimens (either museum specimens or modern individuals) with reliable provenance, which could be designated as types and form the basis for comparative descriptions, are unavailable for most unnamed species (Turvey *et al*. 2019). Efforts to locate additional specimens may fail, despite considerable effort, as *Andrias* populations have undergone catastrophic decline or extirpation across China (Pierson *et al*. 2014, Tapley *et al*. 2015, Turvey *et al*. 2018). Whilst several undescribed *Andrias* species are known to be farmed (Yan *et al*. 2018), husbandry can affect morphology (Martins *et al*. 2013, Hartstone-Rose *et al*. 2014), and selective breeding of farmed *Andrias* can also impact colour (Guo *et al.* 2023); farmed individuals may therefore be atypical and might not constitute appropriate type specimens. Indeed, for morphologically conserved species complexes, diagnostic morphological characters may be insufficient (Angulo and Reichle 2008), and characters traditionally considered diagnostic for particular described *Andrias* species are now known to occur in multiple species (Turvey *et al*. 2019); however, recent morphology-based studies have been able to differentiate between Chinese and Japanese *Andrias* and their hybrids (Hara *et al*. 2023, Takaya *et al*. 2023). Identifying diagnostic characters is further hindered for *Andrias* species as there may now be genetic contamination from translocation and hybridisation in any wild-type individuals detected in surveys (Turvey *et al*. 2018, Yan *et al*. 2018). This hybridisation risk highlights the importance of investigating phylogenetic congruence of both mitochondrial and nuclear markers in potential types, for instance through application of nuclear markers used by Yan *et al*. (2018) or Liang *et al*. (2019), rather than relying solely on mtDNA-based assignment to mitochondrial clades.

The IUCN (1989, 2008) recommends that lethal collection of individuals as holotypes should not normally take place for highly threatened species with very small population sizes. We encourage further investigation of archival collections, especially in Chinese institutions, to try to locate historically wild-caught individuals with known locality data that can be assigned to genetic clades and could serve as types (cf. Waeber *et al*. 2017). Given the urgency of incorporating the Critically Endangered Chinese *Andrias* radiation into a formal taxonomic framework, we also encourage consideration of whether more non-standard approaches are feasible. Remnant surviving *Andrias* populations are sometimes detected when larvae are washed out of karst cave systems (Dai *et al*. 2009, Wang *et al*. 2017, Liang *et al*. 2019), and it is worth considering whether larval samples could serve as valid types, for instance the genotyped specimens reported by Liang *et al*. (2019). Anuran amphibian species have occasionally been described from larval stages (e.g. Grosjean *et al*. 2015), although salamander larvae are morphologically conservative and may lack comparative diagnostic characters. It is also technically possible to describe a species without a dead type, for example using DNA barcodes, living holotypes, or photographs (Jones *et al*. 2005, Gentile and Snell 2009, Meierotto *et al*. 2019, Streicher *et al*. 2020, Sharkey *et al*. 2021); specimen collection therefore does not need to present an obstacle to urgent conservation efforts (O'Donnell *et al*. 2020). However, these approaches are rare and have been heavily criticised by the taxonomic community (Ceríaco *et al*. 2016, Santos *et al*. 2016, Pine and Gutiérrez 2017, Meier *et al*. 2021, Zamani *et al*. 2022a, Zamani *et al*. 2022b). These controversies have increased calls to clarify and standardise appropriate taxonomic protocols for species on the brink of extinction, an approach which we strongly encourage (Nemésio 2009, Krell and Marshall 2017, O'Donnell *et al*. 2020).

Our results demonstrate that all Chinese *Andrias* mitochondrial clades in this study can also be identified using single-gene screening methods, facilitating rapid phylogenetic assignment of genetic samples from future wild-caught individuals for systematic research and conservation. Such efforts should focus on identifying archived specimens of unnamed clades (A, C, D2, F, G2) that could be utilised for taxonomic descriptions, and detecting living wild-type individuals that could form the basis for targeted *in-situ* or *ex-situ* conservation action. Even if future analyses of nuclear data provide new evidence to suggest that some unnamed mitochondrial clades might not constitute valid species, there is still an important argument for conserving them as ESUs (Moritz *et al*. 1994). However, in a practical sense, they are stuck in conservation limbo until they are formally named, and in the time it may take to gather sufficient evidence to achieve this step, some may disappear for good. Chinese *Andrias* populations were first recognised to constitute multiple species over 20 years ago (Murphy *et al*. 2000), and we urge taxonomists to focus more attention on the evolutionary radiation of the world's largest amphibians. We also urge the conservation community to adopt a precautionary approach, and to consider ways to include these undescribed species in national and international conservation policy.

Acknowledgements

Funding was provided by Ocean Park Conservation Foundation Hong Kong (RF201901302247000084), Research England, and the National Natural Science Foundation of China (32060828). We thank Heidi Ma, and appreciate the helpful discussions with colleagues at the 'Conservation Action Plan Workshop for Chinese Giant Salamanders' held in Lanzhou in October 2023.

Author contributions

STT, BT and AAC conceived the ideas; MMM and STT designed the methodology; MMM, STT, ZL and FY collected data; MMM and KH analysed data; MMM, KH, STT, AB and BT interpreted data; STT led the writing of the manuscript. All authors contributed critically to drafts and gave final approval for publication.

Data accessibility

All data used in this study were downloaded from publicly available repositories.

References

An Z, Kutzbach JE, Prell WL et al. Evolution of Asian monsoons and phased uplift of the Himalaya-Tibetan plateau since Late Miocene times. *Nature* 2001;411:62-6.

Angulo A, Reichle S. Acoustic signals, species diagnosis, and species concepts: the case of a new cryptic species of *Leptodactylus* (Amphibia, Anura, Leptodactylidae) from the Chapare region, Bolivia. *Zoological Journal of the Linnean Society* 2008;152:59-77.

Barnes MA, Turner CR. The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics* 2015;17:1-17.

Bickford D, Lohman DJ, Sodhi NS et al. Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution* 2007;22:148-55.

Borzée A, Fong JJ, Nguyen HQ, Jang Y. Large-scale hybridisation as an extinction threat to the Suweon treefrog (Hylidae: *Dryophytes suweonensis*). *Animals* 2020;10:764.

Bouckaert R, Vaughan TG, Barido-Sottani J et al. BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* 2019;15:e1006650.

Boulenger EG. On a new giant salamander, living in the Society's gardens. *Proceedings of the Zoological Society of London* 1924;1924:173-4.

Canastrelli D, Bisconti R, Chiocchio A et al. Climate change promotes hybridisation between deeply divergent species. *PeerJ* 2017;5:e3072.

Carstens BC, Pelletier TA, Reid NM et al. How to fail at species delimitation. *Molecular Ecology* 2013;22:4369-83.

Catenazzi A. State of the world's amphibians. *Annual Review of Environment and Resources* 2015;40:91-119.

CAWCA (China's Aquatic Wildlife Conservation Association). *Bu shiyi kāizhan zengzhi fangliu (fangsheng) de shuisheng shengwu wuzhong jieshao [Aquatic species that are not suitable for breeding and release]*, 2023. Available at : https://mp.weixin.qq.com/s/SCopup9nS1KWOZ10IcO_0w

Ceríaco LM, Gutiérrez EE, Dubois A. Photography-based taxonomy is inadequate, unnecessary, and potentially harmful for biological sciences. *Zootaxa* 2016;4196:435-45.

Chai J, Lu C, Yi M et al. Discovery of a wild, genetically pure Chinese giant salamander creates new conservation opportunities. *Zoological Research* 2022;43:469-80.

Chen Y, Bi J. Biogeography and hotspots of amphibian species in China: implications to reserve selection and conservation. *Current Science* 2007;92:480-9.

Chen M, Mao R, Liang D et al. A reinvestigation of phylogeny and divergence times of Hynobiidae (Amphibia, Caudata) based on 29 nuclear genes. *Molecular Phylogenetics and Evolution* 2015;83:1-6.

Coates DJ, Byrne M, Moritz C. Genetic diversity and conservation units: dealing with the species-population continuum in the age of genomics. *Frontiers in Ecology and Evolution* 2018;6:165.

Coimbra RTF, Winter S, Kumar V et al. Whole-genome analysis of giraffe supports four distinct species. *Current Biology* 2021;31:2929-38.

Costello MJ, May RM, Stork NE. Can we name Earth's species before they go extinct? *Science* 2013;339:413-6.

Cunningham AA, Turvey ST, Zhou F et al. Development of the Chinese giant salamander *Andrias davidianus* farming industry in Shaanxi Province, China: conservation threats and opportunities. *Oryx* 2016;50:265-73.

Dai Q, Wang Y, Liang G. *Conservation Status of Chinese Giant Salamander (Andrias davidianus)*. Chengdu: Chengdu Institute of Biology, 2009.

Davis JI, Nixon KC. Populations, genetic variation, and the delimitation of phylogenetic species. *Systematic Biology* 1992;41:421-35.

Denton RD, Kenyon LJ, Greenwald KR, Gibbs HL. Evolutionary basis of mitonuclear discordance between sister species of mole salamanders (*Ambystoma* sp.). *Molecular Ecology* 2014;23:2811-24.

de Queiroz, K. Species concepts and species delimitation. *Systematic Biology* 2007;56:879-86.

Díaz S, Zafra-Calvo N, Purvis A et al. Set ambitious goals for biodiversity and sustainability. *Science* 2020;370:411-3.

Donath A, Jühling F, Al-Arab M et al. Improved annotation of protein-coding genes boundaries in metazoan mitochondrial genomes. *Nucleic Acids Research* 2019;47:10543-52.

Dubois A, Ohler A, Pyron RA. New concepts and methods for phylogenetic taxonomy and nomenclature in zoology, exemplified by a new ranked cladonomy of recent amphibians (Lissamphibia). *Megataxa* 2021;5:1-738.

Dufresnes C, Mazepa G, Jablonski D et al. Fifteen shades of green: the evolution of *Bufotes* toads revisited. *Molecular Phylogenetics and Evolution* 2019;141:106615.

Dufresnes C, Poyarkov N, Jablonski D. Acknowledging more biodiversity without more species. *Proceedings of the National Academy of Sciences of the USA* 2023;120:e2302424120.

Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 2004;32:1792-7.

Ellepola G, Herath J, Manamendra-Arachchi K et al. Molecular species delimitation of shrub frogs of the genus *Pseudophilautus* (Anura, Rhacophoridae). *Plos ONE* 2021;16:e0258594.

Ely CV, de Loreto Bodignon SA, Trevisan R et al. Implications of poor taxonomy in conservation. *Journal for Nature Conservation* 2017;36:10-3.

Ezard T, Fujisawa T, Barraclough TG. *Splits: species limits by threshold statistics (version 1.0-19)*, 2009. Available at: http://R-Forge.R-project.org/projects/splits/

Fei L, Ye C. *Amphibians of China: Volume I*. Beijing: Science Press, 2017.

Firneno TJ Jr, O'Neill JR, Portik DM, Emery AH, Townsend JH, Fujita MK. Finding complexity in complexes: Assessing the causes of mitonuclear discordance in a problematic species complex of Mesoamerican toads. *Molecular Ecology* 2020;29:3543-59.

Flouri T, Jiao X, Rannala B et al. Species tree inference with BPP using genomic sequences and the multispecies coalescent. *Molecular Biology and Evolution* 2018;35:2585-93.

Flouri T, Rannala B, Yang Z. A tutorial on the use of BPP for species tree estimation and species delimitation. In: Scornavacca C, Delsuc F, Galtier N (eds), *Phylogenetics in the Genomics Era*. Self-published, 2020, 1-16.

Fonseca EM, Duckett DJ, Carstens BC. P2C2M.GMYC: an R package for assessing the utility of the Generalized Mixed Yule Coalescent model. *Methods in Ecology and Evolution* 2021;12:487-93.

Fontaneto D, Herniou EA, Boschetti C et al. Independently evolving species in asexual bdelloid rotifers. *PLoS Biology* 2007;5:914-21.

Freudenstein JV, Broe MB, Folk RA et al. Biodiversity and the species concept—lineages are not enough. *Systematic Biology* 2017;66:644-56.

Fu L, Wang X, Yang S et al. Morphological variation and its environmental correlates in the Taihangshan swelled-vented frog across the Qinling Mountains. *Animals* 2022;12:2328.

Fujisawa T, Barraclough TG. Delimiting species using single-locus data and the Generalized Mixed Yule Coalescent Approach: a revised method and evaluation on simulated data sets. *Systematic Biology* 2013;65:707-24.

Fujita MK, Leaché AD, Burbrink FT et al. Coalescent-based species delimitation in an integrative taxonomy. *Trends in Ecology and Evolution* 2012;27:480-8.

Fukumoto S, Ushimaru A, Minamoto T. A basin-scale application of environmental DNA assessment for rare endemic species and closely related exotic species in rivers: a case study of giant salamanders in Japan. *Journal of Applied Ecology* 2015;52:358-65.

Gao K, Shubin NH. Earliest known crown-group salamanders. *Nature* 2003;422:424-8.

Garnett ST, Christidis L. Taxonomy anarchy hampers conservation. *Nature* 2017;546:25-7.

Gentile G, Snell H. *Conolophus marthae* sp. nov. (Squamata, Iguanidae), a new species of land iguana from the Galápagos archipelago. *Zootaxa* 2009;2201:1-10.

Gong Y, Xu J, Huang S et al. A new species of the giant salamander of the genus *Andrias* from Qimeng, Anhui, China (Amphibia: Cryptorchiidae). *Chinese Journal of Zoology* 2023;58:651-7.

Grosjean S, Bordoloi S, Chuaynkern Y et al. When young are more conspicuous than adults: a new ranid species (Anura: Ranidae) revealed by its tadpole. *Zootaxa* 2015;4058:471-98.

Gu T, Wu H, Yang F et al. Genomic analysis reveals a cryptic pangolin species. *Proceedings of the National Academy of Sciences of the USA* 2023;120:e2304096120.

Gubin YM. Palaeocene salamanders from southern Mongolia. *Paleontological Journal* 1991;1991:91-102.

Guo Y, Wu W, Yang X. Coordinated microRNA/mRNA expression profiles reveal unique skin color regulatory mechanisms in Chinese giant salamander (*Andrias davidianus*). *Animals* 2023;13:1181.

Gutiérrez EE, Helgen KM. Outdated taxonomy blocks conservation. *Nature* 2013;495:314.

Hara S, Nishikawa K, Matsui M et al. Morphological differentiation in giant salamanders, *Andrias japonicus*, *A. davidianus*, and their hybrids (Urodela, Cryptobranchidae), and its taxonomic implications. *Zootaxa* 2023;5369:42-56.

Hartstone-Rose A, Selvey H, Villari JR et al. The three-dimensional morphological effects of captivity. *PLoS ONE* 2014;9:e113437.

Hekkala E, Shirley MH, Amato G et al. An ancient icon reveals new mysteries: mummy DNA resurrects a cryptic species within the Nile crocodile. *Molecular Ecology* 2011;20:4199-215.

Hu J, Li C, Xie F et al. Endemic amphibians and their distribution in China. *Asian Herpetological Research* 2012;3:163-71.

Huang P, Diffendal RF, Yang M. Structural and geomorphological evolution of Huangshan (Yellow Mountain), Anhui Province, China. In: Dort W (ed), *Ter-Qua Symposium Series, Volume 3.* Lawrence, KA: University of Kansas, 2002, 109-24.

Hudson RR. Gene genealogies and coalescent process. In: Futuyma DJ, Antonovics J (eds), *Oxford Surveys in Evolutionary Biology 7*. Oxford: Oxford University Press, 1990, 1-44.

Huson DH, Bryant D. Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* 2006;23:254-67.

Isozaki Y, Aoki K, Nakama T et al. New insights into a subduction-related orogeny: a reappraisal of the geotectonic framework and evolution of the Japanese islands. *Gondwana Research* 2010;18:82-105.

IUCN. *IUCN policy statement on research involving species at risk of extinction*. Gland: International Union for the Conservation of Nature, 1989.

IUCN. *Guidelines regarding research and scientific collecting of threatened species*. Gland: International Union for the Conservation of Nature, 2008.

IUCN. *The IUCN Red List of Threatened Species. Version 2023-1*, 2024. Available at: https://www.iucnredlist.org

IUCN SSC Amphibian Specialist Group. *Andrias davidianus*. The IUCN Red List of Threatened Species, 2023a, e.T179010104A48438418. Available at: https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T179010104A48438418.en

IUCN SSC Amphibian Specialist Group. *Andrias sligoi*. The IUCN Red List of Threatened Species, 2023b, e.T179010130A186541067. Available at: https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T179010130A186541067.en

Jetz W, Pyron RA. The interplay of past diversification and evolutionary isolation with present imperilment across the amphibian tree of life. *Nature Ecology & Evolution* 2018;2:850-8.

Jia J, Anderson JS, Gao K. Middle Jurassic stem hynobiids from China shed light on the evolution of basal salamanders. *iScience* 2021;24:102744.

Jia J, Gao K. A new stem hynobiid salamander (Urodela, Cryptobranchoidea) from the Upper Jurassic (Oxfordian) of Liaoning Province, China. *Journal of Vertebrate Paleontology* 2019;39:e1588285.

Jones T, Ehardt CL, Butynski TM et al. The highland mangabey *Lophocebus kipunji*: a new species of African monkey. *Science* 2005;308:1161-4.

Kearse M, Moir R, Wilson A et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 2012;28:1647-9.

Kingman JFC. Origins of the coalescent: 1974-1982. *Genetics* 2000;156:1461-3.

Köhler J, Vieites DR, Bonett RM et al. New amphibians and global conservation: a boost in species discoveries in a highly endangered vertebrate group. *BioScience* 2005;55:693-6.

Krell FT, Marshall SA. New species described from photographs: Yes? No? Sometimes? A fierce debate and a new declaration of the ICZN. *Insect Systematics and Biodiversity* 2017;1:3-19.

Lanfear R, Calcott B, Kainer D et al. Selecting optimal partitioning schemes for phylogenomic datasets. *BMC Evolutionary Biology* 2014;14:82.

Lanfear R, Frandsen PB, Wright AM et al. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 2017;34:772-3.

Leaché AD, McGuire JA. Phylogenetic relationships of horned lizards (*Phrynosoma*) based on nuclear and mitochondrial data: evidence for a misleading mitochondrial gene tree. *Molecular Phylogenetics and Evolution* 2006;39:628-44.

Li J, Fu C, Lei G. Biogeographical consequences of Cenozoic tectonic events within East Asian margins: a case study of *Hynobius* biogeography. *PLoS ONE* 2011;6:e21506.

Li J, Wei S, Hu M et al. Reflection of paleoclimate oscillations and tectonic events in the phylogeography of moustache toads in southern China. *Journal of Zoology* 2018;305:17-26.

Li Y, Jin M, Zhao J et al. Description of two new species of the genus *Megophrys* (Amphibia: Anura: Megophryidae) from Heishiding Nature Reserve, Fengkai, Guangdong, China, based on molecular and morphological data. *Zootaxa* 2014;3795:449-71.

Li Z, Chen Z, Wan M. Classification and correlation of the Quaternary glacial epoch in the Hengduan (transverse) mountains. *Geological Review* 1991;37:126-32.

Li Z, Yu G, Rao D et al. Phylogeography and demographic history of *Babina pleuraden* (Anura, Ranidae) in southwestern China. *PLoS ONE* 2012;7:e34013.

Liang Z, Chen W, Wang D et al. Phylogeographic patterns and conservation implications of the endangered Chinese giant salamander. *Ecology and Evolution* 2019;9:3879-90.

Lim GS, Balke M, Meier R. Determining species boundaries in a world full of rarity: singletons, species delimitation methods. *Systematic Biology* 2012;61:165-9.

Liu C. *Amphibians of Western China*. Chicago: Chicago Natural History Museum, 1950.

López-Pujol J, Zhang F, Sun H et al. Centres of plant endemism in China: places for survival or for speciation? *Journal of Biogeography* 2011a;38:1267-80.

López-Pujol J, Zhang F, Sun H et al. Mountains of southern China as “plant museums” and “plant cradles”: evolutionary and conservation insights. *Mountain Research and Development* 2011b;31:261-9.

Lu B, Zheng, Y, Murphy RW et al. Coalescence patterns of endemic Tibetan species of stream salamanders (Hynobiidae: *Batrachuperus*). *Molecular Ecology* 2012;21:3308-24.

Mace GM. The role of taxonomy in species conservation. *Philosophical Transactions of the Royal Society B* 2004;359:711-9.

Maddison WP, Knowles LL. Inferring phylogeny despite incomplete lineage sorting. *Systematic Biology* 2006;55:21-30.

Mallo D, Posada D. Multilocus inference of species trees and DNA barcoding. *Philosophical Transactions of the Royal Society B* 2016;371:20150335.

Marshall TL, Chambers EA, Matz MV, Hillis DM. How mitonuclear discordance and geographic variation have confounded species boundaries in a widely studied snake. *Molecular Phylogenetics and Evolution* 2021;162:107194.

Martins FM, Oom MDM, Rebelo R et al. Differential effects of dietary protein on early life-history and morphological traits in natterjack toad (*Epidalea calamita*) tadpoles reared in captivity. *Zoo Biology* 2013;32:457-62.

May R. Taxonomy as destiny. *Nature* 1990;347:129-30.

Meier R, Blaimer B, Buenaventura E et al. A re-analysis of the data in Sharkey et al.'s (2021) minimalist revision reveals that BINs do not deserve names, but BOLD systems needs a stronger commitment to open science. *Cladistics* 2022;38:264-75.

Meierotto S, Sharkey MJ, Janzen DH et al. A revolutionary protocol to describe understudied hyperdiverse taxa and overcome the taxonomic impediment. *Deutsche Entomologische Zeitschrift* 2019;66:119-45.

Moritz C. Defining 'Evolutionarily Significant Units' for conservation. *Trends in Ecology and Evolution* 1994;9:373-5.

Murphy RW, Fu J, Upton DE et al. Genetic variability among endangered Chinese giant salamanders, *Andrias davidianus*. *Molecular Ecology* 2000;9:1539-47.

Murray CM, Russo P, Zorrilla A et al. Divergent morphology among populations of the New Guinea crocodile, *Crocodylus novaeguineae* (Schmidt, 1928): diagnosis of an independent lineage and description of a new species. *Copeia* 2019;107:517-23.

Nadachowska K. Divergence with gene flow – the amphibian perspective. *Herpetological Journal* 2010;20:7-15.

Nee S, May RM, Harvey PH. The reconstructed evolutionary process. *Philosophical Transactions of the Royal Society of London B* 1994;344:305-11.

Nemésio A. On the live holotype of the Galápagos pink land iguana, *Conolophus marthae* Gentile & Snell, 2009 (Squamata: Iguanidae): is it an acceptable exception? *Zootaxa* 2009;2201:21-5.

Nixon KC, Wheeler QD. An amplification of the phylogenetic species concept. *Cladistics* 1990;6:211-23.

O'Donnell DP, Kelly DJ, Analuddin K et al. Adapt taxonomy to conservation goals. *Science* 2020;369:1172.

Padial JM, De la Riva I. A paradigm shift in our view of species drives current trends in biological classification. *Biological Reviews* 2020;96:731-51.

Pan T, Sun Z, Lai X et al. Hidden species diversity in *Pachyhynobius*: a multiple approaches species delimitation with mitogenomes. *Molecular Phylogenetics and Evolution* 2019;137:138-45.

Pierson TW, Yan F, Wang Y et al. A survey for the Chinese giant salamander (*Andrias davidianus*; Blanchard, 1871) in the Qinghai Province. *Amphibian & Reptile Conservation* 2014;8:1-6.

Pine RH, Gutiérrez EE. What is an 'extant' type specimen? Problems arising from naming mammalian species-group taxa without preserved types. *Mammal Review* 2017;48:12-23.

Ping X, Zeng Y. "Guojia zhongdian baohu yesheng dongwu minglu" suo lie wuzhong mingming bianhua ji qi dui yesheng shengwu baohu de yingxiang [Changes in the nomenclature of species listed in the "National Key Protected Wildlife List" and their impact on wildlife conservation]. *Zhongguo Kexue Shengming Kexue* 2020;50:33-43.

Pons J, Barraclough T, Gomez-Zurita J et al. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology* 2006;55:595-610.

R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing, 2021.

Rambaut A, Suchard MA, Xie D et al. *Tracer v.1.6*, 2014. Available at: https://beast.bio.ed.ac.uk/Tracer

Rannula B, Yang Z. Bayes estimation of species divergence times and ancestral population sizes using DNA sequences from multiple loci. *Genetics* 2003;164:1645-56.

Reid NM, Carstens BC. Phylogenetic estimation error can decrease the accuracy of species delimitation: a Bayesian implementation of the general mixed Yule-coalescent model. *BMC Evolutionary Biology* 2012;12:196.

Rong YF, Vasilyan D, Dong LP et al. Revision of *Chunerpeton tianyiense* (Lissamphibia, Caudata): is it a cryptobranchid salamander? *Palaeoworld* 2021;30:708-23.

Ronquist F, Teslenko P, van der Mark DL et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model selection across a large model space. *Systematic Biology* 2012;61:539-42.

Santos CMD, Amorim DS, Klassa B et al. On typeless species and the perils of fast taxonomy. *Systematic Entomology* 2016;41:511-5.

Sharkey MJ, Janzen DH, Hallwachs W et al. Minimalist revision and description of 403 new species in 11 subfamilies of Costa Rican braconid parasitoid wasps, including host records for 219 species. *ZooKeys* 2021;1013:1-665.

Shen X, Liang D, Chen M et al. Enlarged multilocus data set provides surprisingly younger time of origin for the Plethodontidae, the largest family of salamanders. *Systematic Biology* 2016;65:66-81.

Shu G, Liu P, Zhao T et al. Disordered translocation is hastening local extinction of the Chinese giant salamander. *Asian Herpetological Research* 2021;12:271-9.

Shu X, Hou Y, Cheng M, Shu G, Lin X, Wang B, Li C, Song Z, Jiang J, Xie F. Rapid genetic divergence and mitonuclear discordance in the Taliang knobby newt (*Liangshantriton taliangensis*, Salamandridae, Caudata) and their driving forces. *Zoological Research* 2022;43:129-46.

Stamatakis A. "RAxML" Version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014;30:1312-3.

Stamatakis A, Pavlidis P, Kapli P et al. A general delimitation method with applications to phylogenetic placements. Bioinformatics 2013;29:2869-76.

Stewart DJ. A new species of *Arapaima* (Osteoglossomorpha: Osteoglossidae) from the Solimões River, Amazonas State, Brazil. *Copeia* 2013;2013:470-6.

Streicher JW, Sadler R, Loader SP. Amphibian taxonomy: early 21st century case studies. *Journal of Natural History* 2020;54:1-13.

Suk HY, Bae HG, Kim DY et al. Genetic and phylogenetic structure of *Hynobius quelpaertensis*, an endangered endemic salamander species on the Korean Peninsula. *Genes & Genomics* 2019;42:165-78.

Takaya K, Taguchi Y, Ise T. Identification of hybrids between the Japanese giant salamander (*Andrias japonicus*) and Chinese giant salamander (*Andrias* cf. *davidianus*) using deep learning and smartphone images. *Ecology and Evolution* 2023;13:e10698.

Tamashiro RA, White ND, Braun MJ et al. What are the roles of taxon sampling and model fit in tests of cyto-nuclear discordance using avian mitogenomic data? *Molecular Phylogenetics and Evolution* 2019;130:132-42.

Tao F, Wang X, Zheng H et al. Genetic structure and geographic subdivision of four populations of the Chinese giant salamander (*Andrias davidianus*). *Zoological Research* 2005;26:162-7.

Tapley B, Okada S, Redbond J et al. Failure to detect the Chinese giant salamander (*Andrias davidianus*) in Fanjingshan National Nature Reserve, Guizhou Province, China. *Salamandra* 2015;51:206-8.

Tapley B, Turvey ST, Chen S et al. Range-wide decline of Chinese giant salamanders *Andrias* spp. from suitable habitat. *Oryx* 2021;55:373-81.

Thiele KR, Conix S, Pyle RL et al. Towards a global list of accepted species I. Why taxonomists sometimes disagree, and why this matters. *Organisms Diversity & Evolution* 2021;21:615-22.

Thomson SA, Pyle RL, Ahyong ST et al. Taxonomy based on science is necessary for global conservation. *PLoS Biology* 2018;16:e2005075.

Tian S, Kou Y, Zhang Z et al. Phylogeography of *Eomecon chionantha* in subtropical China: the dual roles of the Nanling Mountains as a glacial refugium and a dispersal corridor. *BMC Evolutionary Biology* 2018;18:20.

Tomochika F, Barraclough TG2013. Delimiting species using single-locus data and the generalized mixed yule coalescent (GMYC) approach: a revised method and evaluation on simulated datasets. *Systematic Biology* 2013;62:707-24.

Turvey ST, Chen S, Tapley B et al. Imminent extinction in the wild of the world's largest amphibian. *Current Biology* 2018;28:R592-4.

Turvey ST, Chen S, Tapley B et al. From dirty to delicacy? Changing exploitation in China threatens the world's largest amphibians. *People and Nature* 2021;3:446-56.

Turvey ST, Marr MM, Barnes I et al. Historical museum collections clarify the evolutionary history of cryptic species radiation in the world's largest amphibians. *Ecology and Evolution* 2019;9:10070-84.

Vasilyan D, Böhme M. Pronounced peramorphosis in lissamphibians—*Aviturus exsecratus* (Urodela, Cryptobranchidae) from the Paleocene-Eocene Thermal Maximum of Mongolia*. PLoS ONE* 2012;7:e40665.

Vieites DR, Wollenberg KC, Andreone F et al. Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. *Proceedings of the National Academy of Sciences of the USA* 2009;106:8267-72.

Vogler AP, Monaghan MT. Recent advances in DNA taxonomy*. Journal of Zoological Systematics and Evolutionary Research* 2007;45:1-10.

Waeber PO, Gardner CJ, Lourenço WR et al. On specimen killing in the era of conservation crisis – a quantitative case for modernizing taxonomy and biodiversity inventories. *PLoS ONE* 2017;12:e0183903.

Wandeler P, Hoeck PEA, Keller LF. Back to the future: museum specimens in population genetics. *Trends in Ecology and Evolution* 2007;22:P634-42.

Wang B, Jiang J, Xie F et al. Phylogeographic patterns of mtDNA variation revealed multiple glacial refugia for the frog species *Feirana taihangnica* endemic to the Qinling Mountains. *Journal of Molecular Evolution* 2013;76:112-28.

Wang J, Zhang H, Xie F et al. Genetic bottlenecks of the wild Chinese giant salamander in karst caves. *Asian Herpetological Research* 2017;8:174-83.

Wang X, Zhang K, Wang Z et al. The decline of the Chinese giant salamander *Andrias davidianus* and implications for its conservation. *Oryx* 2004;38:197-202.

Wang Y, Zhao J, Yang J et al. Morphology, molecular genetics, and bioacoustics support two new sympatric *Xenophrys* toads (Amphibia: Anura: Megophryidae) in southeast China. *PLoS ONE* 2014;9:e93075.

Wells KD. *The Ecology and Behavior of Amphibians.* Chicago, IL: University of Chicago Press, 2007.

Wu Y, Wang Y, Hanken J. New species of *Pachytriton* (Caudata: Salamandridae) from the Nanling Mountain Range, southeastern China. *Zootaxa* 2012;3388:1-16.

Xu B, Yang Z. Challenges in species tree estimation under the multispecies coalescent model. *Genetics* 2016;204:1353-68.

Xu J, Yang D, Chen Y et al. Complete mitochondrial genome of a living wild-type Chinese giant salamander *Andrias davidianus* (Amphibia: Cryptobranchidae) in Huangshan. *Mitochondrial DNA Part B: Resources* 2016;1:542-3.

Yan F, Lü J, Zhang B et al. The Chinese giant salamander exemplifies the hidden extinction of cryptic species. *Current Biology* 2018;28:R590-2.

Yang SY, Kim JB, Min MS et al. Genetic and phenetic differentiation among three forms of Korean salamander *Hynobius leechii*. *Korean Journal of Biological Sciences* 1997;1:247-57.

Yang Z. Likelihood and Bayes estimation of ancestral population sizes in hominoids using data from multiple loci. *Genetics* 2002;162:1811-23.

Yang Z, Rannala B. Unguided species delimitation using DNA sequence data from multiple loci. *Molecular Biology and Evolution* 2014;31:3125-35.

Yuan Z, Wu Y, Yan F et al. Comparative multi-locus assessment of modern Asian newts (*Cynops*, *Paramesotriton*, and *Pachytriton*: Salamandridae) in southern China suggests a shared biogeographic history*. Zoological Research* 2022;43:706-18.

Yuan Z, Zhao H, Jiang K et al. Phylogenetic relationships of the genus *Paramesotriton* (Caudata: Salamandridae) with the description of a new species from Qixiling Nature Reserve, Jiangxi, southeastern China and a key to the species. *Asian Herpetological Research* 2014;5:67-79.

Zachos FE, Christidis L, Garnett ST. Mammalian species and the twofold nature of taxonomy: a comment on Taylor et al. 2019. *Mammalia* 2019;84:1-5.

Zamani A, Dal Pos D, Fric ZF et al. The future of zoological taxonomy is integrative, not minimalist. *Systematics and Biodiversity* 2022a;20:1-14.

Zamani A, Fric ZF, Gante HF et al. DNA barcodes on their own are not enough to describe a species. *Systematic Entomology* 2022b;47:385-9.

Zhang J, Kapli P, Pavlidis P et al. A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* 2013;29:2869-76.

Zhang P, Chen Y, Zhou H et al. Phylogeny, evolution and biogeography of Asiatic salamanders (Hynobiidae). *Proceedings of the National Academy of Sciences of the USA* 2006;103:7360-5.

Zhang P, Papenfuss TJ, Wake MH et al. Phylogeny and biogeography of the family Salamandridae (Amphibia: Caudata) inferred from complete mitochondrial genomes. *Molecular Phylogenetics and Evolution* 2008;49:586-97.

Zhao E. *China Red Data Book of Endangered Animals: Amphibia and Reptilia*. Beijing: Science Press, 1998.

Zheng H, Powell CM, An Z et al. Pliocene uplift of the northern Tibetan Plateau. *Geology* 2000;28:715-8.

Zheng Y, Peng R, Kuro-o M et al. Exploring patterns and extent of bias in estimating divergence time from mitochondrial DNA sequence data in a particular lineage: a case study of salamanders (Order Caudata). *Molecular Biology and Evolution* 2011;28:2521-31.

Table 1. Chinese *Andrias* mitogenome sequences used in this study. See source publications for full collection localities and institutional accession details.

GenBank accession no.	**Specimen/ Isolate ID**	**Location (county/region, city, province)**	**Clade name (Yan et al.)**	**Clade name (Liang et al.)**	**Clade name (this study)**	**Referred species**	**Source**
KU131056	GXXA609	Xing'an, Guilin, Guangxi	A	A	A	undescribed	Liang et al. (2019)
KU131051	CQWL481	Huolu, Wulong, Chongqing	D	B	B	*A. sligoi*	Liang et al. (2019)
KU131054	GZGDYX583	Guiding, Qiannan, Guizhou	D	B	B	*A. sligoi*	Liang et al. (2019)
KU131052	HNLS55	Longshan, Xiangxi, Hunan	D	B	B	*A. sligoi*	Liang et al. (2019)
KU131050	HNWMY48	Yongding, Zhangjiajie, Hunan	D	B	B	*A. sligoi*	Liang et al. (2019)
MK177469	NHM 1945.11.7.1	Hong Kong [?originally Guangdong/Guangxi]	D	B	B	*A. sligoi*	Turvey et al. (2019)
MK177465	ZMB 24105	Guangdong or Guangxi	D	B	B	*A. sligoi*	Turvey et al. (2019)
KU131055	GXZY587	Ziyuan, Guilin, Guangxi	U1	C	C	undescribed	Liang et al. (2019)
KU131044	JXJA336	Jing'an, Yichun, Jiangxi	U2	D	D1	*A. jiangxiensis*	Liang et al. (2019)
KU131045	JXJGS352	Jinggangshan, Ji'an, Jiangxi	U2	D	D1	*A. jiangxiensis*	Liang et al. (2019)
KU131046	GDLZ365	Lianzhou, Qingyuan, Guangdong	U2	D	D2	undescribed	Liang et al. (2019)
KX268733	HS16091	Qimen, Huangshan, Anhui	E	E	E	*A. cheni*	Xu et al. (2016)
KU131060	AHHS695	Xiuning, Huangshan, Anhui	E	E	E	*A. cheni*	Liang et al. (2019)
MK177461	ROM 11036	Huangshan, Anhui	E	E	E	*A. cheni*	Turvey et al. (2019)
MK177462	ROM 11037	Huangshan, Anhui	E	E	E	*A. cheni*	Turvey et al. (2019)
MK177463	ROM 11038	Huangshan, Anhui	E	E	E	*A. cheni*	Turvey et al. (2019)
MK177464	ROM 11039	Huangshan, Anhui	E	E	E	*A. cheni*	Turvey et al. (2019)
KU131059	ZJLSQY680	Qingyuan, Lishui, Zhejiang	E	E	E	*A. cheni*	Liang et al. (2019)
KU131042	GSTS240	Qinzhou, Tianshui, Gansu	C	F	F	undescribed	Liang et al. (2019)
KU131043	SCMB244	Mabian, Leshan, Sichuan	B	G	G1	*A. davidianus*	Liang et al. (2019)
MK177467	MCZA 2853	Hongya, Meishan, Sichuan	B	G	G1	*A. davidianus*	Turvey et al. (2019)
MK177468	NHM 1909.7.22.1	Ya'an, Sichuan	B	G	G1	*A. davidianus*	Turvey et al. (2019)
MK177466	NMNH 52409	Ya'an, Sichuan	B	G	G1	*A. davidianus*	Turvey et al. (2019)

KU131040	SXTB202	Taibai, Baoji, Shaanxi	B	G	G2	undescribed	Liang et al. (2019)
KU131058	SXTBYG677	Taibai, Baoji, Shaanxi	B	G	G2	undescribed	Liang et al. (2019)
KU131057	SXSLZS672	Zhashui, Shangluo, Shaanxi	B	G	G2	undescribed	Liang et al. (2019)
KU131047	SXYQLS371	Yuanqu, Yuncheng, Shanxi	B	G	G2	undescribed	Liang et al. (2019)
KU131048	HNJY385	Wangwushan, Jiyuan, Henan	B	G	G2	undescribed	Liang et al. (2019)
KU131061	HNSZSDJ82	Sangzhi, Zhangjiajie, Hunan	B	G	G2	undescribed	Liang et al. (2019)
KU131053	YNYL551	Yiliang, Zhaotong, Yunnan	B	G	G2	undescribed	Liang et al. (2019)

Table 2. Prior settings for population size (θ) and root divergence date (τ) in BPP analysis. Mean θ equates to mean difference between two sequences drawn at random from population. Percent sequence difference in τ represents percent sequence divergence from root to tip of tree.

Run	Theta θ prior	Tau τ prior	Mean θ	Mean τ	τ % sequence difference
1	IG(3,0.0002)	IG(3,003)	0.0001	0.0015	0.15
2		IG(3,03)		0.015	1.5
3	IG(3,0.002)	IG(3,0.0003)	0.001	0.00015	0.015
4		IG(3,003)		0.0015	0.15
5		IG(3,03)		0.015	1.5
6	IG(3,0.02)	IG(3,0.0003)	0.01	0.00015	0.015
7		IG(3,003)		0.0015	0.15

Table 3. Bayesian PTP species delimitation results, showing posterior support for species subdivision and individuals belonging to each group. Both analyses (BEAST tree for Cryptobranchoidea, and Bayesian tree for all-*Andrias*) had similar results for the seven main clades, but only the all-*Andrias* tree recognised species-splits within clades D and G.

BEAST tree (Cryptobranchoidea)	**Accession numbers**	**Posterior probability**	**Bayesian tree (all-*Andrias*)**	**Accession numbers**	**Posterior probability**
A	KU131056	1	A	KU131056	1
B	KU131050, KU131051, KU131052, KU131054, MK177465, MK177469	0.99	B	KU131050, KU131051, KU131052, KU131054, MK177465, MK177469	0.82
C	KU131055	1	C	KU131055	1
D	KU131044, KU131045, KU131046	0.80	D1	KU131044, KU131045	0.94
			D2	KU131046	0.97
E	KU131059, KU131060, KX268733, MK177461, MK177462, MK177463, MK177464	0.99	E	KU131059, KU131060, KX268733, MK177461, MK177462, MK177463, MK177464	0.98
F	KU131042	1	F	KU131042	1
G	KU131040, KU131043, KU131047, KU131048, KU131053, KU131057, KU131058, KU131061, MK177466, MK177467, MK177468	0.95	G1	KU131043, MK177466, MK177467,	0.68
			G2	KU131040, KU131047, KU131048, KU131053, KU131057, KU131058, KU131061	0.37

Table 4. Species delimitation results for BPP analyses based on alignment of Chinese *Andrias* mitogenome sequences. Posterior probabilities shown for seven runs, with highest-supported number of species for each run highlighted in bold.

	Prior		**Number of species**		
	θ	**τ**	**7**	**8**	**9**
Run 1	*IG(3,0.0002)*	*IG(3,0.003)*	0	0.003356	**0.996644**
Run 2		*IG(3,0.03)*	0	0.004368	**0.995632**
Run 3	*IG(3,0.002)*	*IG(3,0.0003)*	0.000698	0.085706	**0.913596**
Run 4		*IG(3,003)*	0.000744	0.093116	**0.906140**
Run 5		*IG(3,03)*	0.000942	0.087926	**0.911128**
Run 6	*IG(3,0.02)*	*IG(3,0.0003)*	0.230316	**0.469020**	0.261808
Run 7		*IG(3,003)*	0.206356	**0.447040**	0.310354

Figure 1. SPLITSTREE network constructed from uncorrected p-distances on Chinese *Andrias* mitogenomes, showing bootstrap support. Names and colours of clades match Liang et al. (2019).

Figure 2. Phylogenetic tree for Chinese *Andrias* mitogenomes showing division into seven highly-supported clades. Topology for ML and Bayesian trees are identical. Branch support for ML bootstrap and Bayesian posterior probabilities are shown.

Figure 3. Time-calibrated BEAST2 mitochondrial genome MCC tree showing Chinese *Andrias* clades and estimated divergence times, and species delimitation results. Vertical bars indicate the highest-supported number of Chinese *Andrias* species recovered from each species delimitation method, alongside a sequence-by-sequence matrix with cell colour indicating probability that sequence pairs are conspecific.

Figure 4. Distribution of ratios of sampled coalescence to Yule rates for bGMYC analysis.

Figure 5. Phylogenetic species tree showing bPTP support for species delimitations within Chinese *Andrias* species complex. Bayesian all-*Andrias* tree and BEAST Cryptobranchoidea tree were both used as bPTP input phylogenies. For species with n>1 samples, samples grouped in red are recovered as conspecific in both analyses, and blue branches indicate further subdivision recovered by the all-*Andrias* tree only. Support values indicate posterior probability that grouped samples are referrable to the same species; where support values differ between phylogenies, support from the Cryptobranchoidea phylogeny is shown in grey.

Figure 6. A, Collection localities for reportedly wild-caught Chinese *Andrias* individuals for which mitogenome data were analysed. Letters and colours correspond to clades in Liang *et al*. (2019). Historical samples lacking precise reported collection localities are not shown. Italicised samples within triangles are likely to represent translocated rather than true wild individuals, as they were collected within the distributions of different clades. **B**, Distribution of Chinese *Andrias* individuals assigned to clades G1 and G2 in mountain regions surrounding Sichuan Basin. Giant salamander silhouette indicates collection locality of *Andrias davidianus* holotype.

Figure 1

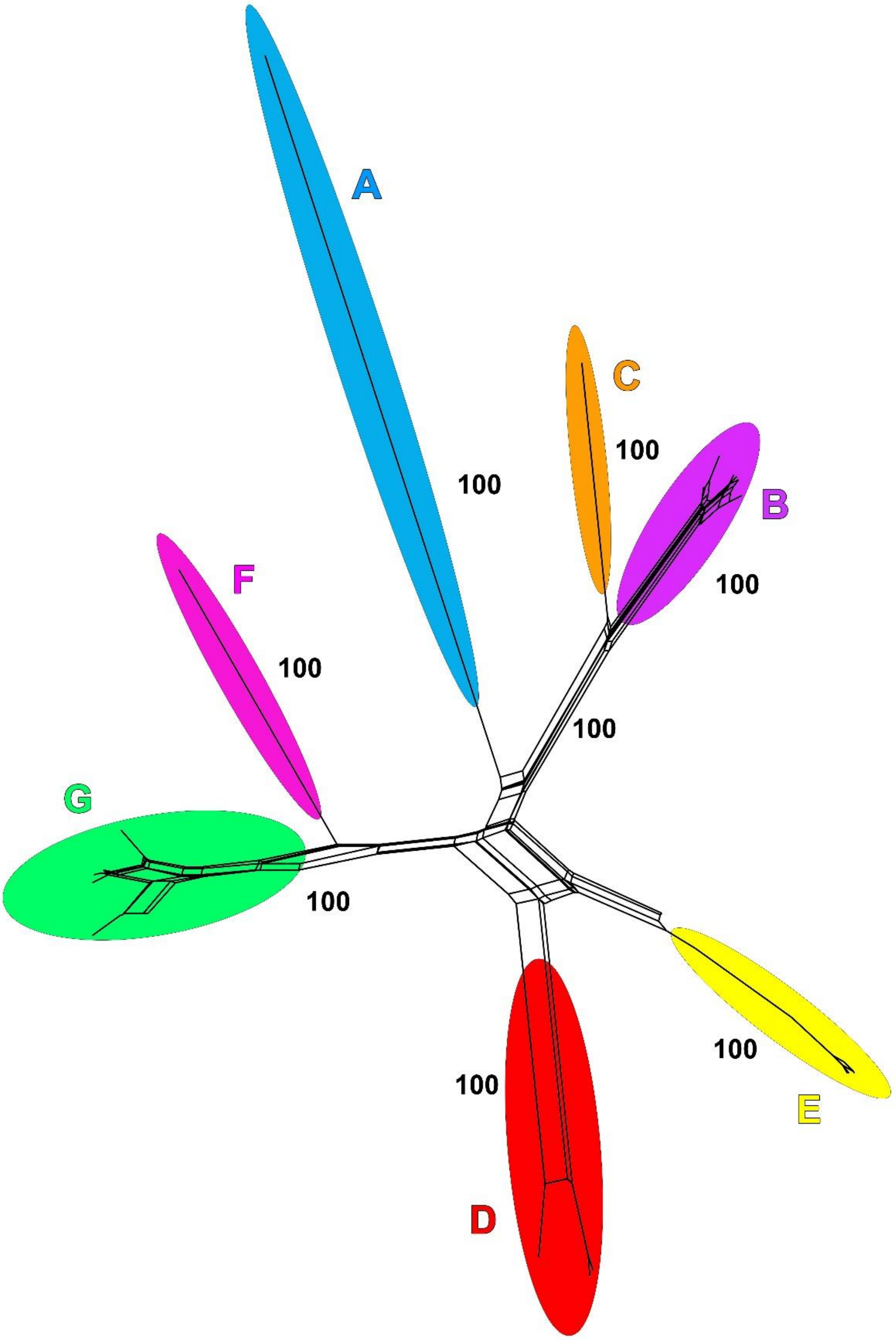


Figure 2

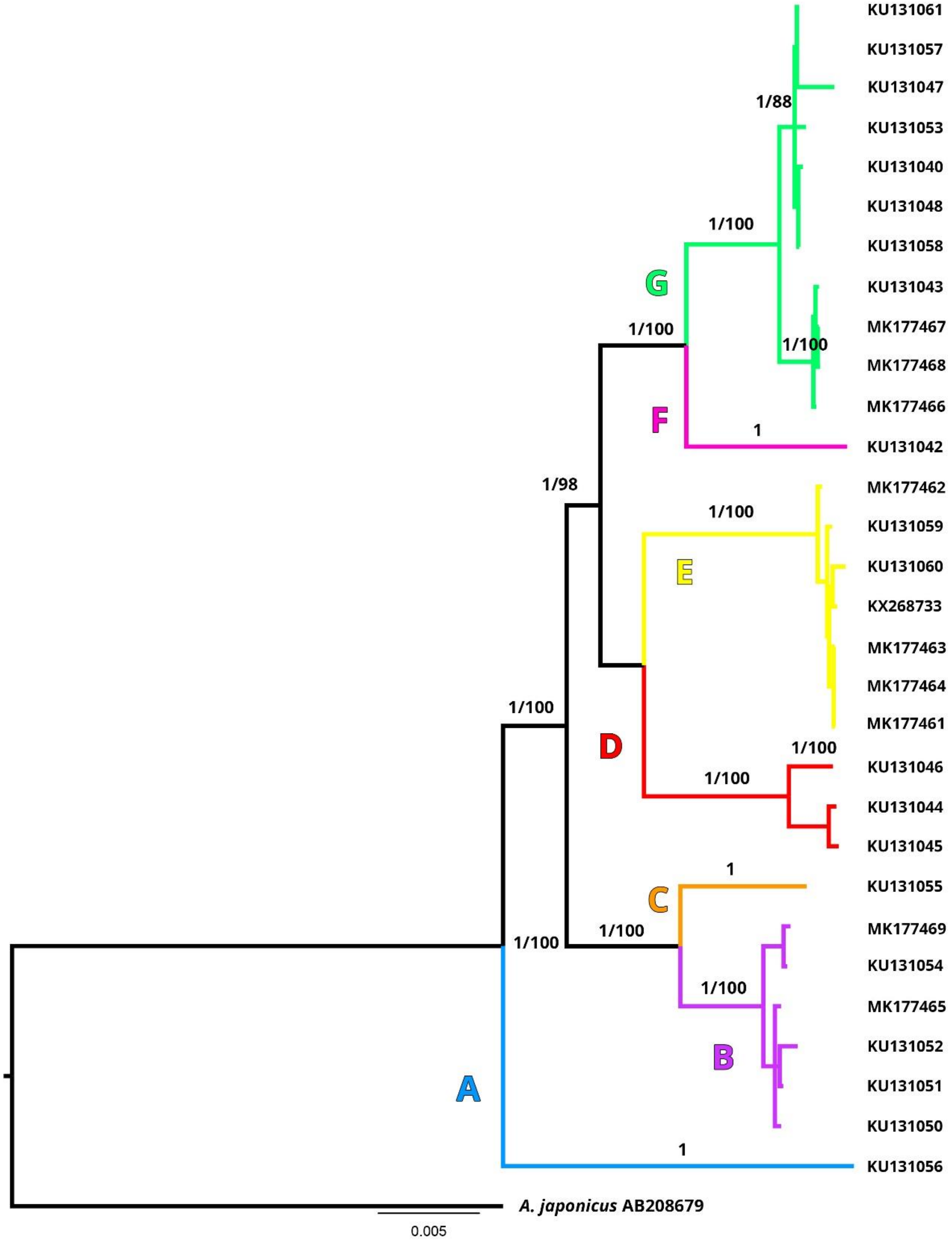

KU131061
KU131057
KU131047
1/88
KU131053
KU131040
KU131048
1/100
KU131058
G
KU131043
1/100
MK177467
1/100
MK177468
MK177466
F
1
KU131042
1/98
MK177462
1/100
KU131059
E
KU131060
KX268733
MK177463
MK177464
1/100
MK177461
D
1/100
KU131046
1/100
KU131044
KU131045
1
KU131055
C
1/100
1/100
MK177469
KU131054
1/100
MK177465
B
KU131052
A
KU131051
KU131050
1
KU131056
A. japonicus AB208679
0.005

Figure 3

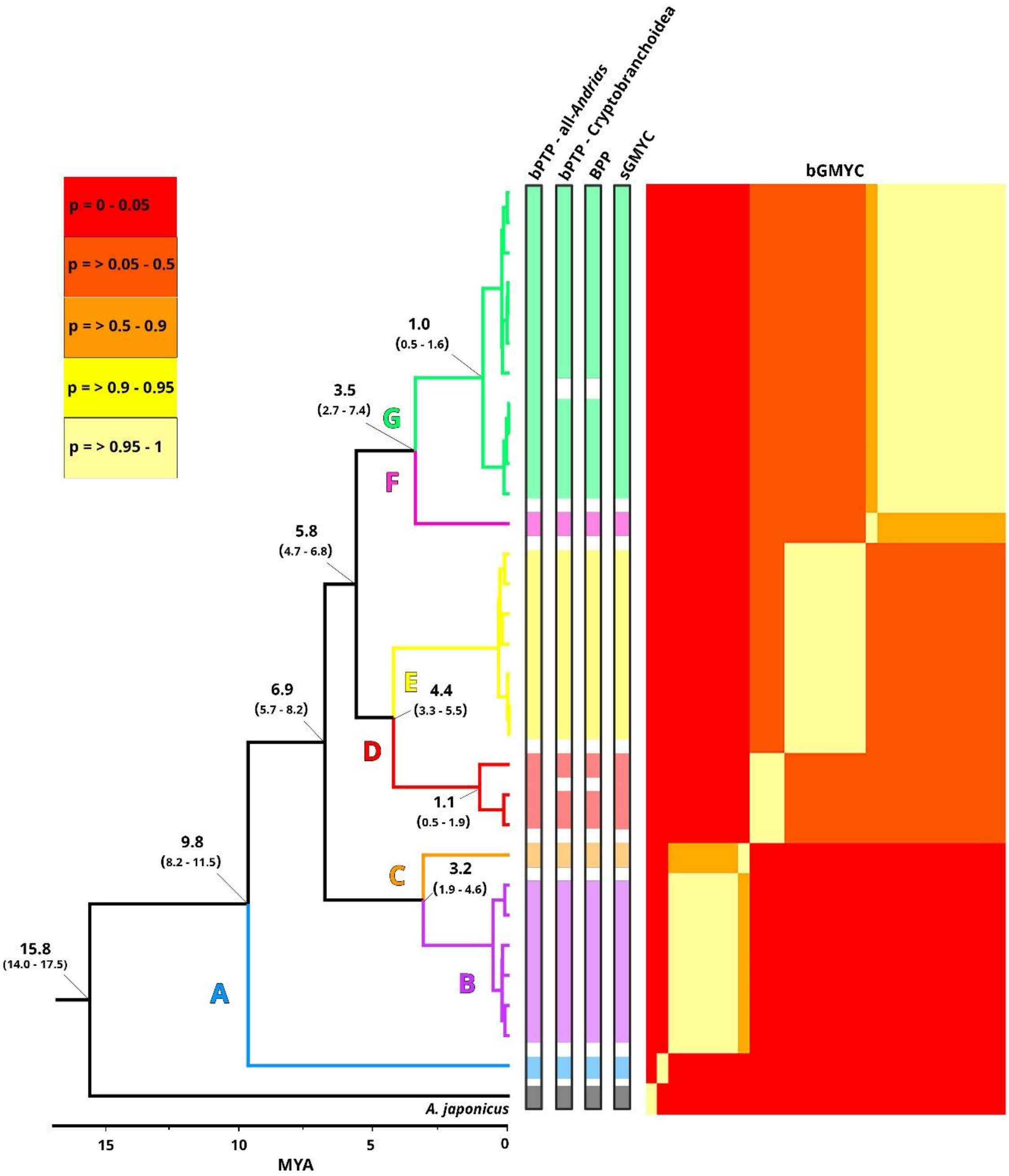
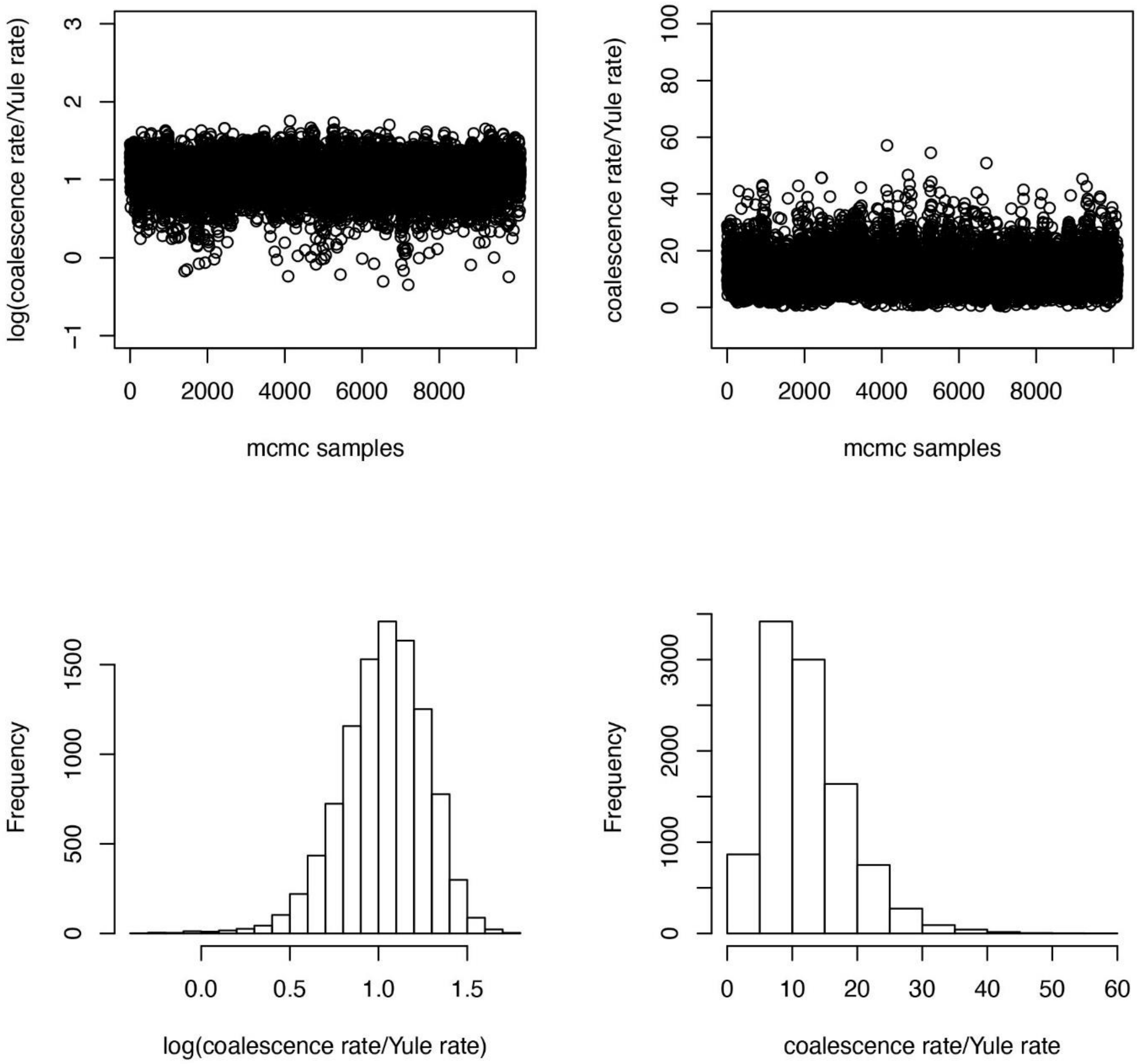
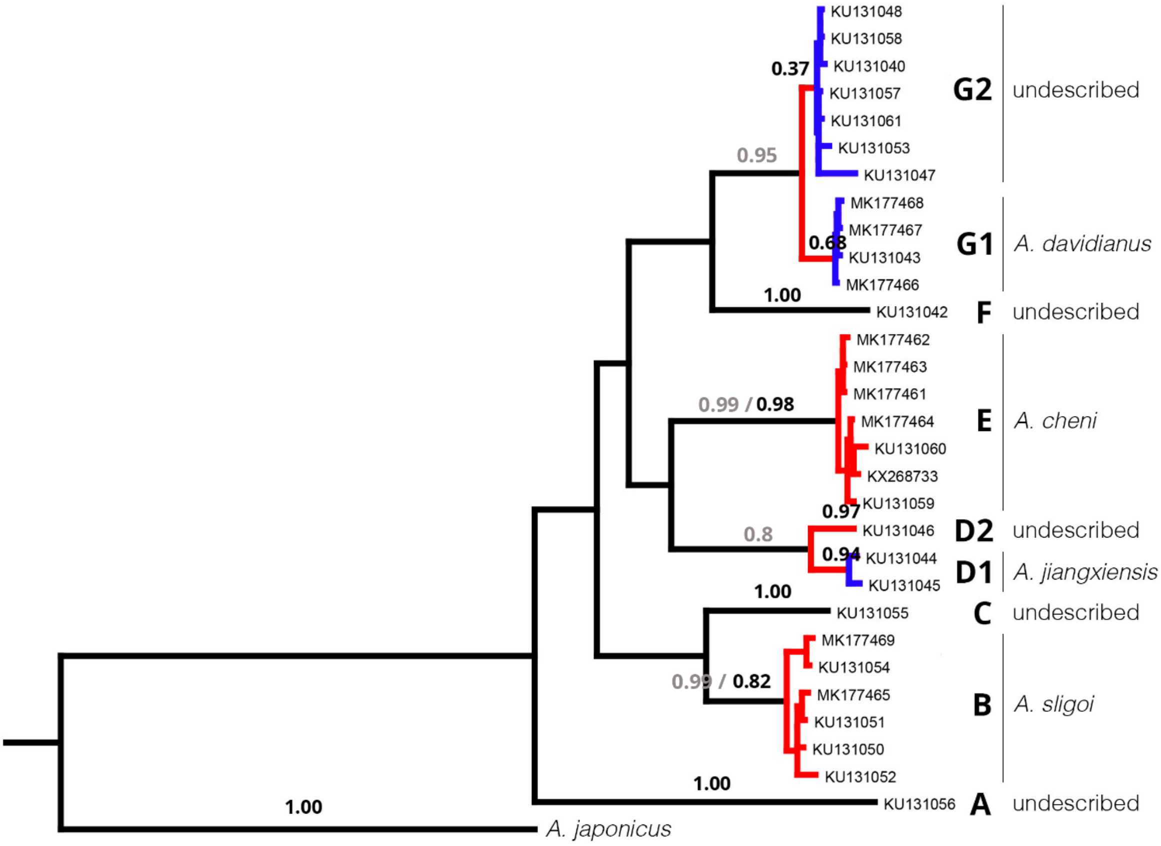

p = 0 - 0.05
p = > 0.05 - 0.5
p = > 0.5 - 0.9
p = > 0.9 - 0.95
p = > 0.95 - 1
bPTP - all-*Andrias*
bPTP - Cryptobranchoidea
BPP
sGMYC
bGMYC
1.0
(0.5 - 1.6)
3.5
(2.7 - 7.4)
G
F
5.8
(4.7 - 6.8)
E
4.4
(3.3 - 5.5)
6.9
(5.7 - 8.2)
D
1.1
(0.5 - 1.9)
9.8
(8.2 - 11.5)
C
3.2
(1.9 - 4.6)
15.8
(14.0 - 17.5)
A
B
A. japonicus
15
10
5
0
MYA

Figure 4



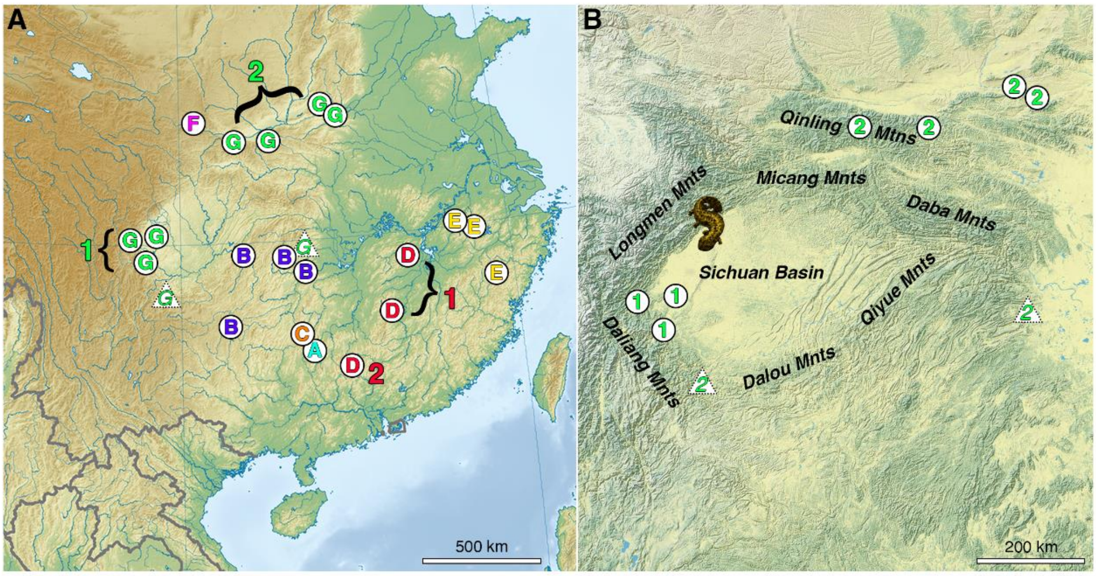
log(coalescence rate/Yule rate)
−1
0
1
2
3
0
2000
4000
6000
8000
mcmc samples
coalescence rate/Yule rate)
0
20
40
60
80
100
0
2000
4000
6000
8000
mcmc samples
Frequency
0
500
1000
1500
0.0
0.5
1.0
1.5
log(coalescence rate/Yule rate)
Frequency
0
1000
2000
3000
0
10
20
30
40
50
60
coalescence rate/Yule rate

Figure 5



KU131048
KU131058
0.37
KU131040
KU131057
G2
undescribed
KU131061
0.95
KU131053
KU131047
MK177468
MK177467
0.68
G1
A. davidianus
KU131043
MK177466
1.00
KU131042
F
undescribed
MK177462
MK177463
MK177461
0.99 / 0.98
MK177464
E
A. cheni
KU131060
KX268733
KU131059
0.97
KU131046
D2
undescribed
0.8
0.94
KU131044
D1
A. jiangxiensis
KU131045
1.00
KU131055
C
undescribed
MK177469
KU131054
0.99 / 0.82
MK177465
B
A. sligoi
KU131051
KU131050
KU131052
1.00
KU131056
A
undescribed
1.00
A. japonicus

Figure 6


A
2
G G
F
G G
1
G G
G
G
B
B G
B
B
E E
D
1
D
E
B
C
A
D 2
500 km
B
2 2
Qinling 2 Mtns 2
Micang Mnts
Longmen Mnts
Daba Mnts
Sichuan Basin
1 1
1
Qiyue Mnts
2
Daliang Mnts
2
Dalou Mnts
200 km